1. TITLE PAGE

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INTEGRATED CLINICAL AND STATISTICAL REPORT (Final Version)

CONFIDENTIAL

OTILONIUM BROMIDE
STUDY NUMBER: Study MeIn/06/OB-20/80/001
EUDRACT NUMBER 2007-001679-12
PHASE: Phase I/II clinical trial

A DOSE-RANGING STUDY OF OTILONIUM BROMIDE IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

Coordinating Investigator:	Dr. Danuta Chmielewska-Wilkoń Gabinet Endoskopii Przewodu Pokarmowego Szewska 4/5 31-009 Krakow Poland
Date first patient entered:	22 October 2007
Date last patient completed:	23 May2008
Name of Sponsor signatory:	Dr. Alessandro Casini, MD
Date of this report:	22 June 2012
Date of any previous reports:	Not Applicable

Design: Double-blind, placebo-controlled, randomized dose-ranging study in 4 parallel groups of 24 (16 female and 8 male) patients each.

This study was performed in compliance with Good Clinical Practices (GCP), including the archiving of essential documents.

APPROVAL SIGNATURES PAGE

STUDY TITLE: A DOSE-RANGING STUDY OF OTILONIUM BROMIDE IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

STUDY NUMBER: MeIn/06/OB-20/80/001

EUDRACT NUMBER 2007-001679-12

By signing this report we certify that it provides a true and accurate record of the conduct of this study and its results.

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Date

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Date

2. SYNOPSIS

Name of Sponsor:	Menarini Industrie Farmaceutiche Riunite Srl.
Name of finished product:	Spasmomen®
Name of active ingredient:	Otilonium Bromide
Title of study:	A DOSE-RANGING STUDY OF OTILONIUM BROMIDE IN PATIENTS WITH IRRITABLE BOWEL SYNDROME
Protocol number:	MeIn/06/OB-20/80/001
Principal Investigator:	Dr. Danuta Chmielewska-Wilkoń Gabinet Endoskopii Przewodu Pokarmowego Szewska 4/5 31-009 Krakow, Poland
Study centre(s):	**4**
Publication (reference):	Not applicable
Studied period (years):	2007 –2008
Phase of development:	Phase I/II clinical trial

Objectives:

Primary - Demonstrate a dose-response relationship of OB - 20 mg, 40 mg, 80 mg and placebo administered t.i.d. for 4 weeks - in treatment-sensitive functional and/or clinical efficacy variables of IBS.

Secondary - The following clinical target variables of efficacy - recorded in the patients' weekly diary - have been analysed:

- The intensity of abdominal discomfort or pain, meteorism or abdominal distension and the severity of diarrhoea and/or constipation have been quantified by five-level score based on the intensity of the discomfort or pain (score 0-4).
- The frequency of abdominal discomfort or pain has been quantified by the five-level score based on the number of episodes (score 0-4).
- Intestinal habits have been identified by one of the following features:
  - constipation
  - diarrhoea
  - alternating
  - regular
- The average number of evacuations during days with evacuation (score 0-3).
- The days without evacuation during the week have been evaluated by the four-level score scale (score 0-3).
- Mucus in the stool, sensation of incomplete evacuation and difficulty of evacuation have been scored during the week by a four-level score scale (score 0-3).
- The consistency and the shape of the stool has been evaluated with the Bristol index.
- Global Discomfort Index (GDI). This index was creates to quantify the clinical efficacy data as a whole.

Safety:

The risk profile was assessed by comparing differences between treatment groups in:

- Incidence of all adverse events
- Withdrawals due to any Adverse Event.
- Serious Adverse Events/hospitalizations.
- Patients' weekly global safety assessment.

Methodology:

Trial design/type: Double-blind, placebo-controlled, randomized dose-ranging study in 4 parallel groups of 24 (16 female and 8 male) patients each.

Study population: 64 female and 32 male Caucasian patients suffering of Irritable Bowel Syndrome.

Number of patients (planned and analysed): 96 planned, 93 analysed.

Diagnosis and main criteria for inclusion:

1. 64 female and 32 male Caucasian patients ≥18 years and ≤ 65 years of age with IBS diagnosed according to the Rome II Criteria 2
2. patients who have at least a 6-month history of IBS, with at least moderate abdominal pain or discomfort occurring on at least 4 days in each of the 4 weeks prior to the study
3. the presence of organic pathology has to be excluded by the following preliminary diagnostic examinations:
   a. accurate anamnesis to exclude in particular the lactase deficiency syndrome (requiring H2-breath tests, when clinically strongly suspected), bowel inflammatory disease, diets or drugs that may cause gastrointestinal symptoms and alvus disturbances
   b. complete physical exam
   c. complete hematology and clinical chemistry
   d. occult blood test in stool (if necessary)
   e. bacteria and parasite stool test (when diarrhoea is the predominant symptom)
   f. complete sigmoidoscopy and/or opaque enema in double contrast (it will be sufficient that these tests have been performed in the last 12 month)
4. written informed consent
5. the use of oral contraceptives or IUD or previous sterilisation required for women of child-bearing potential
6. negative urine pregnancy test required for pre- or premenopausal women (at visit 0).

Main exclusion criteria:

1. patients whose condition cannot be definitely diagnosed as IBS
2. history of intolerance or hypersensitivity to OB
3. alimentary intolerance
4. pregnant or nursing females
5. previous severe abdominal surgery
6. other concomitant diseases which could have a relevant impact on study results
7. any malignancy
8. any concomitant treatment that could affect gastrointestinal motility and function (calcium channel blockers, anticholinergics, prostaglandin drugs, antiacids, prokinetics, meconics, laxatives, antidiarrheals, antidepressives, analgesics, tranquillizers), and which cannot be stopped
9. participation in another clinical study within 2 months prior to enrolment
10. insufficient patient comprehension

Test products, dose and mode of administration:

Film-coated tablets containing Otilonium Bromide were orally administered t.i.d. according to the following treatment schedules:

	08:00	**16:00**	**24:00**
OB I	OB 20 mg	OB 20 mg	OB 20 mg
OB II	OB 40 mg	OB 40 mg	OB 40 mg
OB III	OB 80 mg	OB 80 mg	OB 80 mg
Placebo	OB placebo	OB placebo	OB placebo

Patients were randomly allocated into the four treatment schedules.

Duration of treatment:

4 weeks (28 days)

Criteria for evaluation:

Primary efficacy:

The following functional target variables of efficacy were recorded during the manometric investigations and evaluated:

- Measurement of baseline pressure – tone of anal spyncters
- Voluntary contraction
- Measurement of RAIR
- Sensory assessment during the course of rectal manometry

Secondary efficacy:

The following clinical target variables of efficacy were recorded in the patients' weekly diary and than evaluated:

- The intensity of abdominal discomfort or pain, meteorism or abdominal distension and the severity of diarrhoea and/or constipation were quantified by a five-level score based on the number of episodes (score 0-4).
- The frequency of abdominal discomfort or pain had to be quantified by the four-level score based on the daily number of episodes.
- Intestinal habits identified by one of the following features:
  - constipation: less than 2 evacuations during the week
  - diarrhoea: 3 or more evacuations per day, for at least 5 days
  - alternating: more than 2 days without evacuation together with days with 3 or more evacuations
  - regular: all the conditions not present like constipation or diarrhoea or alternating or more then 2 evacuations during the week.
- The average number of evacuations during days with evacuation quantified by the four-level score based on the daily number of episodes.
- The days without evacuation during the week were evaluated by the four-level score scale based on the number of days without evaluation.
- Mucus in the stool, sensation of incomplete evacuation and difficulty of evacuation were scored during the week by a four-level score scale.
- The consistency and the shape of the stool, evaluated according to Bristol index.

Statistical methods:

For continuous variables, descriptive statistics include for each treatment sequence: mean, standard deviation, median, minimum, maximum, number of non-missing observations. The descriptive statistics for dichotomous or categorical variables are numbers and percentages of each of the scores or categories for each treatment.

The analysis of correlated data arising from repeated measurements (when measurements are assumed to be multivariate and normal) has been studied extensively. However, the assumption of normality might not always be reasonable; for example, different methodology must be used in data analysis when the responses are discrete and correlated.

Sample Size:

The sample size calculation for the study was based on the expected proof of a significant slope of the dose-response curve by linear regression.

The calculations have been performed covering the doses 0 mg (placebo) t.i.d., 20 mg OB t.i.d., 40 mg OB t.i.d. and 80 mg OB t.i.d. Expecting the relation between the standard deviation of the responses and the slope of the dose-response curve not to exceed the value of 250, a sample size of 21 evaluable patients per treatment group (84 evaluable patients) is sufficient to prove significance of the slope at a (one-sided) alpha = 0.025 with the power = 81 %, with 10% drop-out estimated, N = 96.

SUMMARY – CONCLUSIONS

EFFICACY RESULTS:

Analysis of the primary efficacy variables (ano-rectal manometry investigation before and after Otilonium administration) did not show significantly differences between the treatment groups. This was due to the large experimental variability observed.

In consequence of the great variability, observed in the distribution of the Functional target variable of efficacy, the analysis of the Clinical Target Variables of Efficacy was considered as primary objective. This change in the definition of Functional Target Variables of Efficacy were completed before the blind opening phase according to the ICH E9.

This objective was performed measuring the clinical variables of efficacy recorded by patient through in the weekly diary. In particular the "intensity and frequency of abdominal discomfort or pain", the "intestinal habits"; the "number of evacuations and the days without evacuation" and "Mucus in stool incomplete or difficult in evacuation were scored using value scales.

The analysis of these collected data showed that the groups of patients treated with OB 40 mg and 80 mg were significantly different from patients group treated with Placebo or OB 20 mg.

Otherwise, no significant difference was observed comparing the group treated with OB 80 mg with the group treated with and 40 mg OB**.**

SAFETY RESULTS:

The treatment with OB in the range from 20 mg to 80 mg TID was well tolerated, no SAE occurred during the study period.

The number of patients experiencing AEs was low. Most of the AE were of a mild intensity and were not drug related. In conclusion, tolerability of Otilonium Bromide was good and comparable to that observed in Placebo group.

CONCLUSION:

IBS, is a disease of unclear and complex pathophysiology, characterised by abdominal pain, discomfort and altered bowel activity, that affects many individuals worldwide. During trials on a functional disorders as IBS, many confounding factors may appear and complicate the evaluation of the experimental findings. For these reasons in this trial, in order to minimize confounding factors and define IBS baseline values, a study design including a run-in period of 2 weeks, was applied to all to treatment groups. Moreover we introduced the GDI index (Global Discomfort Index) to calculate a value summarizing the data of abdominal discomfort, bloating or pain and number of evacuation observed during the study and the run-in period.

Analysis performed on primary efficacy variables did not show any significant differences between the treatment groups

The analyses performed on clinical variables of efficacy recorded by patient through in the weekly diary ("intensity and frequency of abdominal discomfort or pain", the "intestinal habits", the "number of evacuations" and the "days without evacuation", "Mucus in stool" and incomplete or difficult evacuation) showed the following:

- Patients treated with OB 40 mg and 80 mg were significantly different from patients group treated with Placebo or OB 20 mg.

- No significant difference was observed comparing the group treated with OB 80 mg with the group treated with and 40 mg OB.

- A significant reduction of the GD Index values was observed in the group treated with OB 40 mg ($p<0.01$) and with OB 80 mg ($p< 0.001$). No significant difference was observed for Placebo vs. 20 mg OB group and for 40 mg OB vs. OB 80 mg groups.

The treatment with OB in the range from 20 to 80 mg t.i.d. was well tolerated. No SAE occurred during the study period.

In conclusion we can say that, in patients suffering of bowel irritable syndrome, the treatment with Otilonium Bromide and OB 40 mg in particular, can lead to an improvement of clinical parameters such as: abdominal discomfort, intestinal habits, number of daily evacuations and stool consistency.

Date of the report: 11 June 2012

3. TABLE OF CONTENTS

4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ICD-9	International Classification of Diseases - Version 9
WHO-DRL	World Health Organisation - Drug Reference List
CRF	Case Report Form
IBS	Irritable Bowel Syndrome
OB	Otilonium Bromide
vs	Versus
b.i.d.	Twice a Day
t.i.d.	Three Times a Day
IUD	Intrauterine Device
MedDRA	Medical Dictionary for Regulatory Activities
MI	Motility Index
VAS	Visual Analogue Scale
ITT	Intent-to-treat (population)
PP	Per-protocol (population)
RAIR	Recto-Anal Inhibitory Reflex

5. ETHICS

5.1. INDEPENDENT ETHICS COMMITTEE (IEC)

The protocol, patient information sheet and the statement of informed consent were approved by the Bioethics Committee at the District Chamber of Physicians in Krakow, UL. Krupnicza 11 a. 31123 Krakow.

5.2. ETHICAL CONDUCT OF THE STUDY

The study was conducted in accordance with International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP) and the Declaration of Helsinki.

5.3. PATIENT INFORMATION AND CONSENT

Written informed consent was obtained from each patient prior to screening and before any study procedures were performed.

Sample patient information sheets and consent forms are provided in Appendix 16.1.3.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The CRO in charge of study management, including data entry and statistical analysis, was Medi Service S.r.l.

The study was originally planned to be conducted in 4 centres in Poland. The Study Coordinator was Dr. Danuta Chmielewska-Wilkoń, Gabinet Endoskopii Przewodu Pokarmowego. Appendix 16.1.4 lists the investigators and affiliations.

Study Drug was sent from the Sponsor to the CRO, which was responsible for the distribution. Local laboratories were used for laboratory analysis (haematology, biochemistry and urinalysis).

Medical safety review was performed at the Sponsor's site, with any serious adverse events (SAE) reported to the Clinical Project Manager of the Sponsor. Unblinding was only permitted where deemed necessary on safety grounds.

7. INTRODUCTION

The irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by chronic or recurrent abdominal pain or discomfort, and disturbed defecation. The severity of the disorder ranges from mild to severe and intractable symptoms. IBS is highly prevalent in the general population and is associated with significant disability and health care costs. Prevalence estimates from surveys in the United States and Great Britain indicate that IBS affects 14-24 % of women and 5-19 % of men.[1]

The Rome diagnostic criteria for IBS require the presence for at least 3 months of continuous or recurrent symptoms of abdominal pain or discomfort relieved with defecation, or associated with a change in frequency or consistency of stool [2]. The clinical symptoms of IBS relate to abnormalities in motility and visceral sensation and are influenced by psychosocial factors via the brain-gut axis.[3]

Treatment is based on a combined pharmacological and behavioural approach. Currently, there is no drug that is effective for treatment of all forms and all symptoms of IBS.[3;4]. In view of the evidence of enhanced visceral perception in IBS and the frequent occurrence of pain as a key symptom, it is generally accepted that any agent considered to be of utility in the treatment of IBS should demonstrate effectiveness in the relief of pain.[5]

Otilonium bromide (OB), discovered and developed by Menarini Research SpA [6;7], has a composite mechanism of action which reduces hypermotility and modulates visceral sensation, factors thought to be responsible for pain in IBS.[8;9] OB is a quaternary ammonium derivative with selective spasmolytic action on the gastrointestinal tract, in particular on the colon.[10-12] The predominant action of OB is thought to be the modification of calcium fluxes from intra- and extracellular sites.[8;13-16] Moreover, OB acts as a tachykinin NK2 receptor antagonist and may block NK2 expression on certain visceral afferent nerves.[8;17] It possesses also affinity for muscarinic and PAF receptors and an affinity for L-type calcium channels.[8;18] Pharmacokinetic studies in animals, in human volunteers and in patients clearly showed that OB has a preferential absorption and accumulation in the lower intestine wall, but has a poor systemic absorption.[10;19;22] .In clinical pharmacology studies, performed in healthy subjects and in patients with IBS, the evaluation of gastrointestinal transit, colonic myoelectric activity, colon motility and abdominal pain confirmed OB as a potent spasmolytic drug.[23-27] The spasmolytic activity of OB on the gastrointestinal tract is evident at doses which do not affect gastric secretion, and the drug has been claimed to be devoid of the typical side effects of the antimuscarinic agents used as spasmolytics.[28;29] The extremely good tolerability profile of OB has been proved in animals and human pharmacology and pharmacodynamics studies.[14;16;30;31]

Clinical information

The results of clinical trials in patients with IBS show significant differences after the treatment with OB vs placebo compared to baseline values in parameters such as pain, abdominal distension, motility degree and incomplete emptying.[32-40] Moreover, OB is superior to a high-fiber diet, it seems to have some advantage in comparison to pinaverium and mebeverine and appeared to be equivalent to cimetropium bromide in the treatment of patients with IBS.[41-45]

As reported by Poynard et al. in their meta-analysis in 1994 OB has ranked in the first position among the muscle relaxant agents used in IBS trials using relief of pain as the primary endpoint.[46]

The beneficial role of muscle relaxant agents in the treatment of patients with pain as their dominant symptom is strongly supported by a recent meta-analysis published in 2000 by Jailwala et al.[47]. The recent paper of Czimmer et al. [48] has shown that OB was able to modulate the threshold to the pain in IBS patients but a dose-finding study has not defined so far its optimal dosage in pain modulating activity.

Contra-indications, warnings and special precautions for use

No clinically relevant interactions with other medicaments were observed. At therapeutic doses, the product does not cause side effects; namely it does not cause atropine-like effects. In the animal, otilonium bromide was proven practically devoid of toxicity. Consequently also in the human, no particular overdosage-related problems should appear. In case of overdose a possible symptomatic and support therapy is recommended.

Pharmacokinetics and metabolism

The systemic absorption of Otilonium bromide was suggested to be low after oral administration. Information on the extent of systemic absorption and pharmacokinetic disposition is limited, primary due to a lack of sensitive assay for the determination of Otilonium in biological samples. In studies of ^{14}C Otilonium Bromide in rats, high accumulation of radioactivity was found in intestinal tissue. A trace amount of radioactivity was found only in the liver among various tissues including kidney, lung and muscle in intestinal tissues. Most of the absorbed quota is excreted by faeces 95-97%).

8. STUDY OBJECTIVES

The primary objective of this dose-ranging study was to evaluate the dose-response relationship of OB - 20 mg, 40 mg, 80 mg and placebo administered t.i.d. for 4 weeks - in treatment-sensitive functional and/or clinical efficacy variables of IBS.

For the selection of the recommended effective dose also the safety profile in the different treatment groups has to be considered.

The efficacy target variables have been the responses to colonic motility obtained in ano-rectal manometry investigations and the weekly clinical assessment of IBS in the patients' diary.

Secondary study objective was to evaluate the safety profile considering the physical and laboratory examinations, the incidence of adverse events as well as the patients' global assessment of tolerability. In particular the following clinical target variables of efficacy - recorded in the patients' weekly diary have been analysed:

- The intensity of abdominal discomfort or pain, meteorism or abdominal distension and the severity of diarrhea and/or constipation have been quantified by five-level score based on the intensity of the discomfort or pain (score 0-4)
- The frequency of abdominal discomfort or pain has been quantified by the five-level score based on the number of episodes (score 0-4)
- Intestinal habits have been identified by one of the following features:
  - constipation
  - diarrhoea
  - alternating
  - regular
- The average number of evacuations during days with evacuation (score 0-3)
- The days without evacuation during the week have been evaluated by the four-level score scale (score 0-3)
- Mucus in the stool, sensation of incomplete evacuation and difficulty of evacuation have been scored during the week by a four-level score scale (score 0-3)
- The consistency and the shape of the stool have been evaluated with the Bristol index.

9. INVESTIGATIONAL PLAN

9.1. OVERALL STUDY DESIGN AND PLAN

The study was performed according to the Guidelines for controlled trials and GCP.

Table 1. The study scheme

Visits	-2		-1	0		1	2	3	4
Weeks	Recruitment	-2	-1	Colonic distension / baseline	1	2	3	4	Colonic distension / after treatment
Study arm		**Run-in period**			**Treatment period**				
OB I		No treatment			20 mg OB t.i.d.				
OB II		No treatment			40 mg OB t.i.d.				
OB III		No treatment			80 mg OB t.i.d.				
Placebo		No treatment			Placebo				

The study population has been drawn from male and female out-patients selected according to published diagnostic criteria for IBS (Rome II Criteria). After a 2-week treatment-free run-in period, in all patients still fulfilling the clinical entry criteria, baseline responses to ano-rectal manometry and rectal distension, using a manometric device, have been investigated and baseline assessment of IBS has been recorded.

Patients who report at least moderate symptoms of IBS and demonstrate a clinically relevant degree of colonic hypersensitivity have been - stratified by gender - randomly allocated to either 20 mg OB t.i.d., 40 mg OB t.i.d., 80 mg OB t.i.d. or Placebo for a treatment period of 4 weeks. The blinding of the different dosing regimens has been maintained by double dummy technique.

Patients have recorded their assessment of IBS, adverse events and study drug intake in diaries. At every weekly visit (Visit -1, 0, 1, 2, 3, 4) the patient's diary data have been checked and documented by the investigator in the CRF. Immediately at the end of the treatment period the investigation of responses to distal colonic distension with a rectal balloon device has been repeated.

A flow-chart of the activities performed at each visit is provided in Figure 1.

A copy of the protocol is provided in Appendix 16.1.1. A sample Case Report Form (CRF) is provided in Appendix 16.1.2.

Table 2. Table of assessments.

STUDY FLOW CHART

	Run-in period		Random	Treatment period			End of study
Week	-2 [1]	-1	0	1	2	3	4
Visit	-2	-1 [2]	0 [2]	1 [2]	2 [2]	3 [2]	4 [2]
Informed consent	●						
Patient history	●						
IBS diagnosis	●	● [3]	● [3]				
Inclusion/exclusion criteria	●	● [3]	● [3]				
Physical examination	●		●				●
BP / heart rate	●		●				●
Laboratory measurements	●		●				●
Baseline diary supply	●						
Baseline diary data in CRF		●	●				
Ano rectal investigation			●				●
Randomization			●				
Treatment diary supply			●				
Drug supply			●				
Efficacy assessment				●	●	●	●
Safety assessment				●	●	●	●
Treatment diary data in CRF				●	●	●	●
Compliance assessment				●	●	●	●

[1] Day of recruitment
[2] A visit delay of a few days (3 days at maximum) is acceptable
[3] Confirmation of diagnosis and inclusion/exclusion criteria

9.2. DISCUSSION OF STUDY DESIGN

This was a double-blind, placebo-controlled, randomized dose-ranging study in 4 parallel groups of 24 (16 female and 8 male) patients with Irritable Bowel Syndrome for each group.

Following the screening visit, all patients had to enter a 2-week treatment-free run-in period. Thus, baseline responses to ano-rectal manometry and rectal distension, using a manometric device, were investigated and baseline assessment of IBS was recorded.

Patients who reported at least moderate symptoms of IBS and demonstrated a clinically relevant degree of colonic hypersensitivity were - stratified by gender - randomly allocated to either 20 mg OB t.i.d., 40 mg OB t.i.d., 80 mg OB t.i.d. or Placebo for a treatment period of 4 weeks. The blinding of the different dosing regimens was maintained by double dummy technique.

Patients recorded their assessment of IBS, adverse events and study drug intake in diaries. At every weekly visit (Visit -1, 0, 1, 2, 3 and 4) the patient's diary data was checked and documented by the investigator in the CRF. Immediately at the end of the treatment period the investigation of responses to distal colonic distension with a rectal balloon device was repeated.

9.3. SELECTION OF STUDY POPULATION

9.3.1. Inclusion Criteria

To be eligible for study entry, all patients had to satisfy the following criteria at visit -2 and 0:

a. 64 female and 32 male Caucasian patients > 18 years and < 65 years of age with IBS diagnosed according to the Rome II Criteria[2]

b. patients who have at least a 6-month history of IBS, with at least moderate abdominal pain or discomfort occurring on at least 4 days in each of the 4 weeks prior to the study

c. the presence of organic pathology has to be excluded by the following preliminary diagnostic examinations
   - accurate anamnesis to exclude in particular the lactase deficiency syndrome (requiring H2-breath tests, when clinically strongly suspected), bowel inflammatory disease, diets or drugs that may cause gastrointestinal symptoms and alvus disturbances
   - complete physical exam
   - complete hematology and clinical chemistry
   - occult blood test in stool (if necessary)
   - bacteria and parasite stool test (when diarrhoea is the predominant symptom)
   - complete sigmoidoscopy and/or opaque enema in double contrast (it will be sufficient that these tests have been performed for the same symptoms in the last 12 months; if not, they have to be performed before the run-in period begins)

d. written informed consent

e. the use of oral contraceptives or IUD or previous sterilisation required for women of child-bearing potential

f. negative urine pregnancy test required for pre- or premenopausal women (at visit 0)

9.3.2. Exclusion Criteria

The patient had to be excluded from the study if he/she matched one of the following criteria:

a. patients whose condition cannot be definitely diagnosed as IBS

b. history of intolerance or hypersensitivity to OB

c. alimentary intolerance

d. pregnant or nursing females

e. previous severe abdominal surgery

f. other concomitant diseases which could have a relevant impact on study results

g. any malignancy

h. any concomitant treatment that could affect gastrointestinal motility and function (calcium channel blockers, anticholinergics, prostaglandin drugs, antiacids, prokinetics, meconics, laxatives, antidiarrheals, antidepressives, analgesics, tranquillizers), and which cannot be stopped

i. participation in another clinical study within 2 months prior to enrolment

j. insufficient patient comprehension, co-operation or expression level

9.3.3. Removal of Patients from Therapy or Assessment

The Investigator had to undertake very reasonable effort to maintain a patient within the study protocol. Patients had to be withdrawn from the study in the event of any of the following:

- Withdrawal of patient's consent
- Investigator's or Sponsor's decision
- Therapeutic failure requiring urgent additional medication (lack of efficacy)
- Use of any of the concomitant medications specified as not permitted in section 9.4.7, during the whole course of the study
- Occurrence of an AE which was considered intolerable by the patient or the Investigator, or of a concomitant disease not allowed by protocol
- Lack of patient compliance (<80% or >120% of prescribed study medication)
- Patient death
- Occurrence of any exclusion criterion

Patients who withdrew prematurely from the study had to be asked to attend the study site for withdrawal assessments as soon as possible after the last dose of study medication. A withdrawn patient could not re-enter the study and the medication designated for this patient could not be given to any other patient. Withdrawn patients could not be replaced during this study.

In case of premature discontinuation from the study a complete physical examination had to be performed as far as possible with regard to the patient's health and as far as necessary with regard to safety aspects and validity of study results.

9.4. TREATMENTS

After a 2-week treatment-free run-in period, patients who reported at least moderate symptoms of IBS and demonstrate a clinically relevant degree of colonic hypersensitivity were stratified by gender and randomly allocated to either 20 mg OB t.i.d., 40 mg OB t.i.d., 80 mg OB t.i.d. or Placebo for a treatment period of 4 weeks. The blinding of the different dosing regimens were maintained by double dummy technique. Table 3 details the treatment schedules that was followed during the study:

Table 3. Treatment schedule.

	08:00	**16:00**	**24:00**
OB I	OB 20 mg	OB 20 mg	OB 20 mg
OB II	OB 40 mg	OB 40 mg	OB 40 mg
OB III	OB 80 mg	OB 80 mg	OB 80 mg
Placebo	OB placebo	OB placebo	OB placebo

9.4.1. Start of treatment

The patient started the intake of trial medication the next morning after his visit 0, i.e. the day after his baseline assessment of responses to colonic distension.

9.4.2. Duration of the treatment

Treatment covered a period of 4 weeks (28 days). If distal colonic manometry could not be performed immediately the next day (day 29), the patient continued taking the drug for a period of three days maximum using the provided reserve blisters.

9.4.3. Treatments Administered

Regular treatment in this study covered 4 weeks (28 days).

9.4.4. Drug administration on the day after treatment colonic distension

The morning dose on the day of assessment of responses to colonic distension immediately after the end of the 4-week treatment period was taken at the investigator's site under the supervision of medical staff and documented by the investigator in the CRF.

9.4.5. Identity of Investigational Products

All test products adhered to good manufacturing practices (GMP).

Otilonium Bromide

Otilonium bromide medications consisted of blistered tablets containing 20 mg or 40 mg. The blisters were packaged in boxes as detailed in the next paragraphs and sections.

Pharmaceutical characteristic of the formulations were the following:

Otilonium Bromide Tablets
Product Code: OB 20
Formulation: film-coated tablets for oral administration, immediate release
Strength 20 mg

Product Code: OB 40
Formulation: film-coated tablets for oral administration, immediate release
Strength 40 mg

Placebo The inactive placebo of used for the wash-out phase was tablet with the same appearance as the active medication but filled with an inactive placebo mixture

Placebo Tablets
Product Code: OB placebo
Compound: no active compound
Formulation: film-coated tablets for oral administration, immediate release

Batch Number by Treatment and Drug Supply

Batch numbers are reported in following table:

Drug	Formulation	Batch	Expiry date	Manufacturer
Placebo	tablets	TFE0516	04/2010	A. Menarini M.L. & S.
Otilonium Bromide 20 mg	tablets	TFN0604	09/2010	A. Menarini M.L. & S.
Otilonium Bromide 40 mg	tablets	072	12/2009	A. Menarini M.L. & S.

Storage and disposition of supplies

At the Investigator's site the study medication had to be stored, in accordance with the manufacturer's instructions displayed on the label, in a separate, securely locked, limited-access storage area at a temperature no higher than 30°C. Unused drug supplies had to be returned to the Sponsor after completion of the trial. All supplies had to be accounted for at the end of the study.

Packaging and labelling information

Study Medication Kit

For each patient had to be arranged a study medication kit with 4 medication boxes for the treatment along the foresee four weeks.

As the randomization has to be stratified by gender, the labelling of the study medication for female and male patients were different by colour.

In each box were present a "flag label" containing all the information about drug and study protocol according to guidelines and actual legislation.

The removable part of the label were stick on the CRF's pages in order to demonstrate the affective use of blisters by the patient.

Medication Boxes for the Treatment Period

Each medication were contained 7 labelled blisters for one week of the treatment period plus 3 labelled blister for 3 additional days. (total 10 blisters)

Each blister contained 6 tablets that is the complete study medication for one treatment day.

The patient was instructed to take always two tablets at the same time at scheduled hours (i.e. 8.00, 16.00, 24.00)

Drug accountability

The patients had to be asked to return their study medication container with the 4 medication boxes– including all unused and used (empty) blisters - to the study centre at the visits 1 to 4 for drug accountability. In addition, the entries in the patient diaries had to be checked for consistency with the number of tablets returned. The patient's entries in the diary concerning the study medication intake had to be transcribed into the CRF by the investigator.

9.4.6. Method of Assigning Patients to Treatment Groups

Patients considered eligible for the study, after signing their informed consent, had to receive a screening number and begin a washout period with placebo. After the washout period, patients satisfying all inclusion criteria and no exclusion criteria had to be randomly allocated to one of the four treatment schedules as depicted in table 3.

Each centre received quantity of the study drugs based on enrolment rate.

The randomization list was specific for each centre and generated by a computer according to a specific program. A randomised, per centre, block allocation was used to assign each patient to her/his treatment group

The patient randomisation numbers had to be allocated sequentially by centre, in the order in which the patients entered the study.

Each Investigator had to retain the code-break envelopes for her/his patients, which had to be kept in a secure place. Further copies of the code breaks had to be held by the CRO and the Sponsor.

A code-break envelope had only to be opened by the Investigator (or deputy) when knowledge of patient's randomisation group would have affected the management of an adverse experience or the subsequent continuation of the study. Except in emergency, the code-break should not have been opened without discussion with a medical representative of the Sponsor. The reasons for breaking the code had to be fully documented in the CRF. Envelopes for the remaining subjects had not to be opened. All code-break envelopes had to be collected by the study monitor at the end of the study and returned to the Sponsor. A statement that the correct procedure was followed had to be documented in the study file.

A detailed description of the randomisation method and the full randomisation list are provided in Appendix 16.1.7.

9.4.7. Selection of Doses in the Study

OB doses were selected in order to verify on "in treatment-sensitive functional and/or clinical efficacy variables of IBS" the possible dose-response relationship of OB when administered at 20 mg, 40 mg, and 80 mg t.i.d. for 4 weeks.

9.4.8. Selection and Timing of Dose for Each Patient

All study medications, including placebo, had to be taken according the following scheme:

	08:00	**16:00**	**24:00**
OB I	OB 20 mg + Placebo	OB 20 mg + Placebo	OB 20 mg + Placebo
OB II	OB 40 mg + Placebo	OB 40 mg + Placebo	OB 40 mg + Placebo
OB III	OB 40 mg + OB 40 mg	OB 40 mg + OB 40 mg	OB 40 mg + OB 40 mg
Placebo	OB placebo + OB Placebo	OB placebo + OB Placebo	OB placebo + OB Placebo

At each scheduled time point of dosage administration, the patients were instructed to take two tablets at the same time. According to the dosage schedule, the patients in all 4 study arms received their study medication 3 times a day.

9.4.9. Blinding

All the Otilonium Bromide (OB) tablets administered were identical in size, shape, and colour.

Each OB tablet contained 20 mg or 40 mg of OB active ingredient.

The OB-matching placebo tablets were identical in appearance, colour and weight.

Unblinding

The drug blinding code was sealed and retained by the Investigator.

Exclusively for safety reason, the Investigator could break the treatment code for an individual patient. In this case the Investigator must record in detail on the CRF (Adverse Event section) the reasons for code breaking. dating and signing the appropriate section of sealed blinding code immediatly after the event.

In no other instance the code could be broken without prior consultation with the clinical monitor.

9.4.10. Prior and Concomitant Therapy

No other treatment of signs and symptoms of IBS or concomitant treatment that could affect gastrointestinal motility and function have been allowed as long as the patient takes part in the study. If any additional IBS medication or concomitant treatment that could affect gastrointestinal motility and function is definitely necessary at any time point, the study has been prematurely terminated for this patient.

Concomitant treatment for other chronic concomitant diseases has been allowed only if the patient has been regularly taking such medication for at least two months prior to the start of the study, and should not be changed during the study. In this case the generic name, dose, duration of treatment and reason for prescription has been documented.

The patients should not change their dietary habits during the whole course of the study.

9.4.11. Treatment Compliance

Treatment compliance had to be calculated at each study visit by the Investigator counting returned tablets and using the following formula:

$$\text{Compliance (\%)} = \frac{\text{number of tablet taken}}{\text{number of tablet which should have been taken}} \times 100$$

According to study protocol, the compliance was considered insufficient in the following case:

- a score under 80% in the treatment weeks 1 and 2.
- a score under 90% in the treatment weeks 3.
- a score under 100% in the treatment weeks 4.

Treatment compliance must be recorded in the CRF by the Investigator, patients with an insufficient compliance were drop-out from the study.

9.5. EFFICACY AND SAFETY VARIABLES

9.5.1. Efficacy and Safety Measurements Assessed and Flow Chart

A flow-chart of study procedures and visit is reported in figure 1. Procedures carried out for each visit are detailed in the following paragraphs.

Each patient had to be submitted to 7 medical visits during the study (run-in: 2 week of treatment-free period; double-blind phase: consisting of 4 weeks of treatment period). Each medical visit could be performed some days before or after the planned date.

Screening visit (visit -2, week -2)

According to EMEA guidelines and in order to avoid the introduction of possible confounding factors two weeks of run-in period was foreseen for all the patients considered eligible for the study. The Investigator had to carefully follow the patient during the run-in period, and thus an intermediate visit, 7 days after the screening visit, was foreseen. During visit -2 patients had to be selected on the basis of the inclusion/exclusion criteria. The Investigator had to exhaustively inform the patient about the study's aims and modality of execution. He had to leave the patient sufficient time to obtain any clarification he/she needed and to obtain the signed informed consent.
After signing the informed consent, patients had to be considered enrolled and the procedures required by the protocol could start. The enrolled patient had to receive a provisional number (screening number), composed by patient's sequence number, e.g. 001 (centre no. 1, patient's sequence no. 001). Collected data had to be recorded in the CRF.

During the visit the Investigator had to perform the following activities:

(1) assign the provisional number (CRF number)
(2) collect post and present patient medical history
(3) perform physical examination, recording of blood pressure and heart rate (pre-study)
(4) checking for inclusion/exclusion criteria
(5) perform IBS diagnosis according to the Rome II criteria
(6) perform laboratory examination (pre-study)
(7) inform about the nature of the dose-ranging trial (with special attention to the functional evaluation of responses to ano-rectal motility and distension in the manometric investigations) and any risks involved
(8) supply baseline diary explaining the baseline diary compilation to the patient.
(10) compile the CRF pages linked to his/her screening visit data
(11) arrange with patient the date of his/her next visit. (visit -1)

The Investigator had to give to the patient a letter for the General Practitioner, outlining the characteristics of the study in which patient agreed to participate. Patients were invited to contact the Investigator in the event of the onset of disturbances of any kind.

Visit -1 (week -1)

This visit was been planned 1 week after Visit -2. During this visit the Investigator had to verify the following.

(1) original considerations of "IBS" diagnosis
(2) the inclusion/exclusion criteria
(3) patient's diary entries of the week -2 (run-in period) and transfer diary data on patient's CRF
(4) to schedule the date of next visit (Visit 0) providing patients with written information concerning the preparation and the course of the baseline manometric investigation.

Visit 0 (Day 0 – Baseline Manometric investigation)

At the end of the run-in period, one week after visit –1, patients had to undergo visit 0 or randomisation visit and baseline ano-rectal investigation.
During this visit the Investigator had to verify the following.

(1) original considerations of "IBS" diagnosis
(2) the inclusion/exclusion criteria
(3) patient's diary entries recoded during the week -1 and transfer diary data on patient's CRF.

Then the investigator realized the following activities

(4) physical examination, recording of blood pressure and heart rate (pre-treatment)
(5) laboratory measurements (pre-treatment)
(6) preparation of the manometric investigations (enema)
(7) baseline manometric investigations

Patients satisfying all inclusion criteria and no exclusion criteria had to receive a randomisation number, stratified by gender, identifying the assignment to one of the four treatment groups.

(8) supply of trial medication to the patient:

Each patient received a study medication container having 4 medication boxes for the treatment of 4 weeks and 1 reserve medication box. The patients were asked to bring their complete study medication container (including empty medication boxes and empty blisters) to the study centre at every treatment visit 1 to 4 for drug accountability.

At the end of the visit the investigator:

(9) provided the patient with study diary treatment explaining him/her its compilation

(10) planned the next visit date

The data recorded at visit 0 had to be considered baseline values. Few days of visit delay were acceptable (3 days at maximum).

Visit 1, visit2 and Visit 3 (Days 8, 15, 22 of the treatment period)

These visits were been planned after 1, 2 and 3 weeks of active treatment respectively and had to include:

(1) verification of patient's diary entries for week 1, 2, 3 of the treatment period, respectively, and transfer diary data on patient's CRF.

(2) check of AEs and of any concomitant treatments (adverse event form).

(3) assessment of drug accountability and compliance. The returned unused drug had to be counted and compliance verified.

(4) arrangement of next visit

(5) only at visit 3, Investigator provide patients with written information concerning the preparation and the course of the post treatment visit and manometric investigation.

Few days of visit delay were acceptable (3 days at maximum).

Visit 4 (Day 29 – post treatment manometric investigation)

The conclusive visit was planned after 4 weeks of active treatment and had to include:

(1) verification of patient's diary entries for week 4 of the treatment period, and transfer diary data on patient's CRF.

(2) check of AEs and of any concomitant treatments (adverse event form).

(3) assessment of drug accountability and compliance. The returned unused drug had to be counted and compliance verified.

(4) physical examination, recording of blood pressure and heart rate (post-treatment)

(5) laboratory measurements (post-treatment)

In case of abnormal findings observed in laboratory examination at visit 4, or premature (prior to visit 4) treatment termination, laboratory examination were repeated 8 - 12 days later.

(6) preparation of the manometric investigations (enema)

(7) post treatment manometric investigations (responses of ano-rectal motility and distension in the manometric investigations)
Patients satisfying all inclusion criteria and no exclusion criteria had to receive a randomisation number, stratified by gender, identifying the assignment to one of the four treatment groups.

Maximum three days of "visit 4" delay were accepted. In case there was a delay in the post-treatment manometric investigation, drug intake and its documentation were strictly continued (using the patient's reserve medication box).

9.5.1.1. Efficacy Measurements Assessed

Functional Evaluation: ano-rectal motility and responses to Distension

After the 2-week treatment-free run-in period, the patients baseline responses to rectal motility and distension was investigated, using a manometric system. At the end of the 4 weeks treatment period the manometric investigation was repeated.

Schedule of Manometric Investigations and Preparation of the Patient

To avoid any influence of potential diurnal changes in colonic sensitivity and motility, all patient-related activities were scheduled for the same times of both investigational days.

Patients were admitted to the clinical research unit - after a 12 h fast - in the early morning at 6:30 a.m. at each of the two days scheduled for visit 0 and visit 4.

The evening before visits 0 and 4, the patients undergo a 1-liter tap-water enema. The patients remained fasting until the manometric investigation was completed.

The patients remained fasting until the manometric investigation was completed.

During visit 4, the morning medication dose was taken from the blister "Medication on the day of after-treatment colonic manometry" under the supervision of medical staff at 7:00 a.m.

Because OB is a locally acting drug, and considering colonic transit time after drug intake; the efficacy assessment by rectal manometry was assessed about 5 hours later last drug dose ingestion.

Waiting for manometric investigation, the morning hours were used to perform all other (non-manometric) activities scheduled during visit 0 or visit 4 and to carefully inform the patient about the course of the manometric investigation. All test measures had to be reported onto the CRF

Ano-rectal Manometry - Method and Definitions

The manometric testing was realized starting at 12:00 a.m.

To reduce pelvic hydrostatic pressure patients were placed in a left lateral decubitus position (Sims' position).

The manometric probe was attached to the perfusion system and the perfusion was opened.

The perfusion rate of the probe was set to 0.5 ml per minute (i.e. 10 drops of distilled water per minute for each channel); the perfusion rate was checked before every examination.

The probe was placed at the anus level and the pressure recorded was kept as zero.

After stopping perfusion, the manometric open ended-tips water perfused probe, with a latex balloon at the top, was inserted through the anus, into the rectum so that the 4 radial open ended-tips were placed into the rectum.

After 5 minutes with the perfusion closed, manometric investigations were started.

The first step was to calculate the pressure profile of the anal canal.

To do this, the probe was slowly extracted (approximately 1 mm per second) until the radial open-ended tips are outside the anal canal.

Then, the probe was inserted again and this manoeuvre was repeated.

Then the probe was placed in the point of maximum pressure and fixed to the patient.

The following parameters were calculated as detailed below

(1) Measurement of baseline pressure – tone of anal spyncters
The baseline pressure is defined as the mean pressure of 5 minutes period with the patients lying on left side (Sims' position). The basal pressure is the expression of the basal tone of both internal and external anal sphincter.

(2) Voluntary contraction
The patients were asked to "squeeze" the muscles of external anal sphincter; in other words, the patients had to contract the external anal sphincter for at least 20 seconds. This manoeuvre was repeated 3 times and calculated the mean of the maximal pressure recorded.

(3) Measurement of RAIR
Recto-Anal Inhibitory Reflex (RAIR) is an automatic reflex caused by a distension of the rectal wall that provokes an inhibition of the tone of the internal anal sphincter.
The balloon was manually and rapidly inflated with 10 ml of air for 10 seconds, and then deflated. In the case the RAIR does not appear, the procedure was repeated with 20, 40, 60, 80, 100, 140, 180 end 200 ml of air, until the reflex was evoked.

(4) Sensory assessment during the course of rectal manometry
The balloon was rapidly inflated with 10 ml of air for 10 seconds, and then deflated.
The patient had to refer if he/she had a sensation of air in the rectum, desire to defecate, urgency to defecate or discomfort.

Thereafter, this measurement was be repeated inflating the balloon with 20, 40, 60, 80, 100, 140, 180 end 200 ml of air, until discomfort is reached.

Clinical Evaluation. Assessment by the patient

The patient had to fill a weekly diary card at the end of each week in the 2-weeks run-in period to record baseline values, and in the 4-weeks treatment period for efficacy assessment.
During the treatment period, patients had to give a weekly global efficacy assessment by answering the question whether, or not, they had obtained - compared to the baseline period - adequate relief of their IBS pain and discomfort during the previous 7 days.
Moreover, the patients had to evaluate in detail signs and symptoms of IBS like abdominal pain and discomfort, meteorism and abdominal distension as well as alvus characteristics by 12 different endpoints to be evaluated by the following assessment.

The intensity of abdominal discomfort or pain, meteorism or abdominal distension and the severity of diarrhoea and/or constipation had to be quantified by the following score scale:

score 0: absence of symptom
score 1: presence of mild symptom (they don't interferes with normal everyday life)
score 2: moderate symptom (it almost never interferes with normal everyday life)
score 3: severe symptom (it almost always interferes with normal everyday life)
score 4: very severe symptom (it strongly interferes with everyday life, interruption of work etc)

The frequency of abdominal discomfort or pain had to be quantified by the four-level score based on the number of episodes:

score 0: no episodes during the week
score 1: 1 to 2 episodes during the week
score 2: 3 to 4 episodes during the week
score 3: 5 to 7 episodes during the week
score 4: 8 or more episodes during the week

Intestinal habits had to be identified by one of the following features:

constipation: less than 2 evacuations during the week
diarrhoea: 3 or more than 3 evacuations per day, for at least 5 days
alternating: more than 2 days without evacuation together with days with 3 or more evacuations
regular: all the conditions not present like constipation or diarrhoea or alternating or more then 2 evacuations during the week.

The average number of evacuations during days with evacuation:

score 0: 1 or less per day
score 1: 2 per day
score 2: 3 or 4 per day
score 3: 5 or more per day

The days without evacuation during the week were be evaluated by the four-level score scale:

score 0: 0 to 1 day without evacuation
score 1: 2 to 3 days without evacuation
score 2: 4 to 5 days without evacuation
score 3: 6 to 7 days without evacuation

Mucus in the stool, sensation of incomplete evacuation and difficulty of evacuation had to be scored during the week by a four-level score scale:

score 0: never
score 1: sometimes (less than 40% of evacuation)
score 2: frequently (equal or more than 40% of evacuation)
score 3: constantly (every evacuation)

The consistency and the shape of the stool had to be evaluated with the Bristol index (a gradual 7 points scoring scale (from 1 = watery, to 7 = very hard):

Type 1 = separate hard lumps, like nuts (hard to pass)

Type 2 = sausage shaped but lumpy

Type 3 = like a sausage but with cracks on its surface

Type 4 = like a sausage or snake, smooth and soft

Type 5 = soft blobs with clear cut edges (passed easily)

Type 6 = fluffy pieces with ragged edges, a mushy stool

Type 7 = watery, no solid pieces

“Global Discomfort Index (GDI)" was created in order to evaluate the clinical efficacy data as a whole. The GDI index is calculated according the following formula:

$$GDI = \frac{\text{(Daily frequency of the abdominal discomfort, bloating or pain) * (N° evacuations)}}{\text{GDI Mean (Screening Period)}} * 100$$

Where:
GDI Mean = Mean of the Efficacy Index in the 14 days before randomization (Screening Period).

9.5.1.2. Safety Measurements Assessed

Target Variables of Safety

The risk profile in the different treatment groups was evaluated by the following target variables of:

- clinically relevant different findings in pre- and post-treatment physical examinations
- clinically relevant different findings in pre- and post-treatment laboratory examinations
- adverse events
- patients' weekly global safety assessment

9.5.1.2.1. Adverse Events

Eliciting and Documenting AEs

It was the responsibility of the Investigator to document all AEs occurring during the study. An AE had to include any noxious, pathological or unintended change in anatomical, physiological or metabolic functions as indicated by physical signs, symptoms and/or laboratory changes whether associated with the study drug and whether or not considered drug related. This had to include an exacerbation of pre-existing conditions or events, intercurrent illnesses, drug interaction or the significant worsening of the indication under investigation that was not recorded elsewhere in the CRF under specific efficacy assessments. Anticipated day-to-day fluctuations of pre-existing conditions, including the disease under study, that did not represent a clinically significant exacerbation or worsening had not to be considered AEs.

Patient entry into the study had to be defined as the time at which informed consent was obtained (this had to be before any protocol-specific diagnostic procedures or interventions). All subsequent AEs had to be reported regardless of whether or not they were considered to be drug related. Trial medication had to include both the drug under evaluation and placebo used during the run-in phase.

The occurrence of AEs had to be determined objectively, or subjectively, by asking the patient a non-leading question, for example "Have you experienced or are you experiencing any new or changed symptoms since we last asked/since your last visit?". AEs had to be reported on the appropriate page of the CRF. Any available information or diagnostic measure had to be attached to the CRF.

Assessment of Causality

Every effort had to be made by the Investigator to explain each AE and assess its relationship to study drug treatment, if any. Causality had to be assessed using the following categories:

Certainly related: a clinical event, including a laboratory test abnormality, occurring in a plausible time relation to the administration of the drug, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

Probably related: a clinical event, including a laboratory test abnormality, with a reasonable time relation to the administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information (AE reappearance after drug reintroduction) is not required to fulfil this definition.

Possibly related: a clinical event, including a laboratory test abnormality, with a reasonable time relation to the administration of the drug, could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal (dechallenge) may be lacking or unclear.

Unlikely related: other drugs, chemicals or underlying disease provide plausible explanations and/or the temporal relation to the administration of the drug makes a causal relation improbable

Not related: existence of a clear alternative explanation, and/or unreasonable temporal relationship between drug and event, and/or non plausibility

Unassessable: it cannot be judged, because of the information is insufficient or contradictory and cannot be supplemented or verified.

Any AE considered at least possibly related to study medication by the Investigator and/or the sponsor had to be considered an adverse drug reaction (ADR).

Assessment of Severity

Each AE had to be assigned a category of severity, determined by the Investigator or reported to her/him by the patient. The assessment of severity had to be made irrespective of drug relationship or seriousness of the experience and had to be evaluated according to the following scale:

Mild: an AE which was easily tolerated by the patient, caused minimal discomfort and did not interfere with everyday routine activities.

Moderate: an AE sufficiently discomforting to interfere with everyday routine activities.

Severe: an AE preventing performance of everyday routine activities.

If there was a change in severity of an AE, it had to be recorded as a separate event.

Follow-up of AEs

All Investigators had to follow-up patients with AEs until the event has been resolved or until, in the opinion of the Investigator, the event was stabilised or determined to be chronic (even if this occurred after the date of therapy discontinuation or study end). Details of AE resolution had to be documented in the CRF.

9.5.1.2.2. Serious Adverse Events

Definition of a serious adverse event (SAE)

A SAE is defined as any event:

1) Fatal (resulting in death)
2) Life threatening: an AE was life threatening if the patient was at immediate risk of death from the event as it occurred, i.e. it had not to include a reaction that might have caused death if it had occurred in a more serious form
3) Resulting in hospitalisation or prolonging an existing hospital stay: complications occurring during hospitalisation are AEs and are SAEs if they caused prolongation of the current hospitalisation. Hospitalisation for elective treatment of a pre-existing non-worsening condition had not, however, to be considered an AE. The details of such hospitalisations had to be recorded on the medical history/physical examination page of the CRF
4) Disabling or incapacitating: an AE was incapacitating or disabling if it resulted in a substantial and/or permanent patient's disability
5) Being a congenital anomaly or birth defect
6) Being is another medically important condition that may jeopardise the patient or may require intervention to prevent one of the outcomes listed above

In addition, medical and scientific judgement was required to decide if prompt notification was mandatory in other situations, i.e. any event which the Investigator regarded as serious that did not strictly meet the criteria above but may have jeopardised the patient or required intervention to prevent one of the outcomes listed above, or which would suggest any significant hazard, contraindication, side effect or precaution that may be associated with the use of the drug.

Definition of non serious AE/ADR

Is an AE or ADR which does not fulfil the conditions for the definition of SAE.

Definition of an unexpected AE/ADR

Any AE that was not expected, i.e. one that had not been reported as expected in the protocol or the Investigator's Brochure or the Summary of Product Characteristics (SmPC), either from previous clinical studies or the pre-clinical studies.

Reporting of SAEs

The Investigator had to report any SAE (whether or not thought to be related to the investigational drug) sending by fax the CRF-AE recording pages **no later than 24 hours** after knowledge of the event to the Study Medical Expert.

All SAEs had to be followed-up until the outcome.

Any information and supporting documentation that became available (copies of laboratory reports, tests, procedures, autopsy evidence of the cause of death, etc.) had to be provided by the Investigator to the Study Medical Expert and to the CRO, accompanied by additional written reports as soon as possible.

The Investigator had also to comply with the local applicable obligation(s) on the reporting of ADR to the local concerned Regulatory Authority/ Ethic Committee

Medical Assistance

If there was a need to discuss any medical issues concerning a SAE, then the medical monitor had to be contacted.

Regulatory Responsibility

The Sponsor is responsible for notifying the regulatory authorities about the safety of a new drug. Fatal and life threatening unexpected cases must be notified no later than 7 days, while other unexpected serious cases must be notified no later than 15 days.
Therefore, the investigator must promptly notify SAEs so that reporting timelines could be met and also to ensure ethical responsibilities towards the safety of other patients were met. The Sponsor must inform all participating Investigators and Ethics Committees of serious unexpected drug related events associated with the use of this drug. The Investigators must adhere to local requirements of the IEC with regard to reporting these events locally.

9.5.1.2.3. Routine Laboratory Tests

The laboratory tests must be performed on the enrolment day (visit -2) and repeated immediately before both the beginning of the treatment period, at visit 0, and after the end of the treatment period, at visit 4.

Relevant abnormal findings in the patient's laboratory test at visit -2 or at visit 0 must exclude the patient from the study.

If there are abnormal findings in the laboratory test at visit 4 or at premature treatment termination prior to visit 4 a repeat laboratory test is required 8 - 12 days later

The full laboratory testing must include:

- Haematology: (1) Hemoglobin (Hb), Hematocrit (Hk), Erythrocytes (RBC), Leukocytes (WBC) including differential count, Platelet count.
- Biochemistry: Hemoglobin (Hb), Hematocrit (Hk), Erythrocytes (RBC), Leukocytes (WBC) including differential count, Platelet count; Creatinine, Fasting glucose, Total bilirubin, Direct bilirubin, SGOT, SGPT, Gamma-GT, LDH, AP, Urea (BUN), Total cholesterol, Triglycerides,

Total protein, Sodium, Potassium, Chloride, Phosphate, MCV, MCH, MCHC, VES, PCR, IgE, AGA, EMA, Anti Tg, pH

- Urinalysis: pH, Nitrite, Protein, Glucose, Ketones, Urobilinogen, Blood, Bilirubin; Pregnancy test (only at visit 0 in pre- or premenopausal women).

Samples must be taken with the fasted patient.

The results of all known laboratory tests required by the protocol had to be recorded onto the CRF.

All clinically serious abnormal laboratory tests, occurring during the study, had to be repeated with appropriate medical management until they returned either to normal or to a level deemed acceptable by the Investigator and the clinical monitor or a clinical diagnosis of intercurrent illness was confirmed.

9.5.1.2.4. Physical examination

The physical examination must be performed on the enrolling day (visit -2) and repeated immediately before the beginning of the treatment period (visit 0) and immediately after the end of the treatment period (visit 4). It must include the heart rate record, systolic and diastolic blood pressure and a status of body system (physical investigations referring to diagnosis of IBS).

9.5.2. Appropriateness of Measurements

The assessments made in this study are standard, widely used and generally recognised as reliable, accurate and relevant.

9.6. DATA QUALITY ASSURANCE

Study monitoring

Monitoring of this study had to follow procedures developed by the CRO in order to comply with GCP and to ensure international acceptability of the study data. Therefore the Investigator had to make the records available to the CRO, upon request at reasonable times. CRF had to be checked for completeness and clarity.

The study had to be monitored at regular intervals during the whole study duration. Data verification had to be done by direct comparison with source documents in case of patient's respective consent, always giving due consideration to data protection and medical confidentiality. The following had to be reviewed during monitoring visits:

- Compliance with the protocol
- Consent procedure
- Source document
- AE procedures
- Storage and accountability of materials

After completion of the study, all unused study materials had to be collected by the clinical monitor. However, the Investigator had to retain the patient identification codes (i.e. unit code, trial code identification and randomisation number) for at least 15 years after completion or discontinuation of

the study. Other source documents such as patient files, clinical case notes, had to be retained for the maximum period of time allowed by the hospital, but no less than 15 years after the completion or discontinuation of the study. The Investigator had also to retain her/his copies of the CRF and other study documentation for this period of time.

Audit and inspection

This study could be subjected to audit by the sponsor or their representatives, or by Regulatory Authorities. The audits could be undertaken to check compliance with the requirements of GCP and the ICH Guidelines. The Investigator had to allow the representative of the sponsor or of the Regulatory Authorities:

- To inspect the site, facilities and material used for the study
- To meet all persons involved in the study
- To have access to study data and source documents
- To consult all the documents relevant to the study

No audits were required and performed during this study.

Data recording

All data obtained during the study had to be documented by the Investigator or her/his designate on the CRF provided by the Sponsor. This also applied to the data for those patients who, after having consented to participate, underwent baseline examinations required for inclusion into the study but whom, because a criterion for exclusion was met or for other reasons, were not included in the study.

To ensure legibility the CRF had to be filled out in block capitals with a black ball-point pen. Any correction on the CRF had to be carried out by the Investigator or her/his designate. A single stroke had to be drawn through the original entry. The correction had to be dated and initialled. Incorrect entries had to be covered with correcting fluid, or obliterated, or made illegible in any way.

Even if there were no changes from a previous examination, in the interest of completeness of data acquisition, the questions repeated in each section of the CRF had to be answered in full. A reasonable explanation had to be given by the Investigator for all missing data.

The CRF had to be completed and, after being signed by the Investigator, had to be returned to the Sponsor immediately after termination of the individual final examination, according to an arrangement with the clinical monitor.

Data Management

The CRO had to be responsible for activities associated with the data management of this study. This had to include producing a CRF, setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. All data had to be double entered into a Data Management System, the second entry verifying the first. Automated checks had to be made against the data to ensure completeness and consistency. The database and check programmes had to be validated prior to implementation. Setting of database data entry and data analysis was performed by Medi Service-Italia.

Missing or inconsistent data had to be queried in writing to the Investigator for clarification. Subsequent modifications to the database had to be documented.

The complete database had to have a random sample review of transcription of data from CRFs to the database (quality control) to ensure acceptable quality, according to the standard procedures of data management. Any findings had to be reviewed and the database had to be amended as necessary.

9.7. STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

9.7.1. Statistical and Analytical Plans

9.7.1.1. Data Sets Analyzed

Three sets of Analysis Population are defined:

Intent-to-treat (ITT): The intent-to-treat population is a subset of the safety population with reasonable adherence to essential features of the protocol. The ITT-population covers all randomized patients, who

(1) have the disease under study (IBS)
(2) have taken at least one dosage of study medication
(3) provide follow-up data for the target parameters.

Per-Protocol (PP): The per-protocol population is a subset of the intent-to-treat patients with strict adherence to critical features of the protocol. Major protocol violations in this sense are:

(1) non-confirmation of diagnosis of IBS
(2) violation of inclusion/exclusion criteria or of eligibility criteria for randomization
(3) insufficient compliance:
    - in the treatment weeks 1 and 2 study drug intake < 80 %
    - in the treatment week 3 study drug intake < 90 %
    - in treatment week 4 until visit 4 study drug intake < 100 %
(4) unblinding (e.g. open emergency envelope)
(5) other than study drug treatment of IBS
(6) missing or insufficient diary data for the clinical assessment of IBS
(7) missing or insufficient data of response to manometric investigations.

The nature of and reasons for these protocol violations must be defined and documented in the statistical analysis plan.

The definition of the per-protocol population has been reviewed and possibly updated as a result of

the blind review of the study data, and had to be finalized before breaking the blind.

Safety: all patients randomised to either sequence and having not positively refused to receive the study treatment they have been randomised to, for whom it cannot be excluded that have taken at least one dose of study drug.

9.7.1.2. Demographics and Baseline Characteristics

Descriptive statistics were provided for all demographic data and baseline characteristics, for the intention-to-treat population (ITT Population).

- Distribution of gender and age by treatment group.
- Baseline values (mean ± SD) for all efficacy parameters by treatment group.
- The general medical history for each treatment group by tables and listings.
- Baseline physical examination and previous medications will be described by tables and listings.

The demographic and baseline characteristics are presented in Table 1.4-1.7 and in Listing 3 and 4.

9.7.1.3. Primary Efficacy Variables

Functional Target Variables of Efficacy (Primary Endpoint)

The following functional target variables of efficacy - recorded during the manometric investigations - have been analysed:

- Measurement of baseline pressure - tone of anal sphincters

The baseline pressure is defined as the mean pressure of 5 minutes period with the patients lying on left side (Sims' position). The basal pressure is the expression of the basal tone of both internal and external anal sphincter.

- Voluntary contraction

The patients have been asked to "squeeze" the muscles of external anal sphincter; in other words, the patients had to contract the external anal sphincter for at least 20 seconds. This manoeuvre has been repeated 3 times and the mean of the maximal pressure recorded has been calculated.

- Measurement of RAIR

Recto-Anal Inhibitory Reflex (RAIR) is an automatic reflex caused by a distension of the rectal wall that provokes an inhibition of the tone of the internal anal sphincter.

- Sensory assessment during the course of rectal manometry

The balloon has been rapidly inflated with 10 ml of air for 10 seconds, and then deflated.

9.7.1.4. Secondary efficacy analysis

The following clinical target variables of efficacy - recorded in the patients' weekly diary - have been analysed:

- The intensity of abdominal discomfort or pain, meteorism or abdominal distension and the severity of diarrhoea and/or constipation were to be quantified by the following score scale:

score 0: absence of symptom
score 1: presence of mild symptom (they don't interferes with normal everyday life)
score 2: moderate symptom (it almost never interferes with normal everyday life)

score 3: severe symptom (it almost always interferes with normal everyday life)
score 4: very severe symptom (it strongly interferes with everyday life)

- The frequency of abdominal discomfort or pain had to be quantified by the four-level score based on the number of episodes:

score 0: no episodes during the week
score 1: 1 to 2 episodes during the week
score 2: 3 to 4 episodes during the week
score 3: 5 to 7 episodes during the week
score 4: 8 or more episodes during the week

- Intestinal habits had to be identified by one of the following features:

constipation: less than 2 evacuations during the week
diarrhoea: 3 or more than 3 evacuations per day, for at least 5 days
alternating: more than 2 days without evacuation together with days with 3 or more evacuations
regular: all the conditions not present like constipation or diarrhoea or alternating or more then 2 evacuations during the week.

- The average number of evacuations during days with evacuation. :

score 0: 1 or less per day
score 1: 2 per day
score 2: 3 or 4 per day
score 3: 5 or more per day

- The days without evacuation during the week have been evaluated by the four-level score scale:

score 0: 0 to 1 day without evacuation
score 1: 2 to 3 days without evacuation
score 2: 4 to 5 days without evacuation
score 3: 6 to 7 days without evacuation

- Mucus in the stool, sensation of incomplete evacuation and difficulty of evacuation had to be scored during the week by a four-level score scale:

score 0: never
score 1: sometimes (less than 40% of evacuation)
score 2: frequently (equal or more than 40% of evacuation)
score 3: constantly (every evacuation)

- The consistency and the shape of the stool had to be evaluated with the Bristol index:

Type 2 = sausage shaped but lumpy
Type 3 = like a sausage but with cracks on its surface
Type 4 = like a sausage or snake, smooth and soft
Type 5 = soft blobs with clear cut edges (passed easily)
Type 6 = fluffy pieces with ragged edges, a mushy stool
Type 7 = watery, no solid pieces

9.7.1.5. Compliance

Treatment compliance descriptive analysis computed as described in section 9.4.8 is summarised in the following Table, 4.

Table 4. Compliance descriptive analysis.

Compliance (%)		
Treatment	**Mean**	**Std Dev**
placebo	93.8222	12.14048
OB 20mg	93.6833	12.23447
OB 40mg	91.2789	22.39798
OB 80mg	92.2762	20.14664
Total	92.7963	16.91788

9.7.1.6. Safety Variables

All patients enrolled were considered in assessing tolerability (Safety Population). Safety was assessed by comparing differences between treatment groups in:

- Incidence of all Adverse Events.
- Serious Adverse Events/hospitalizations.
- Withdrawals due to any Adverse Event.

Any alternative statistical methods that may be used as appropriate in the statistical analysis of the data and the reason for any departure from the statistical plan stated above was fully documented.

Adverse Events

Adverse events were coded using the MedDRA (version 10.1) classification with reference to system organ class and preferred terms. The number (%) of subjects with adverse events was presented by treatment group.

The number of subjects reporting at least one adverse event was summarized for each treatment group.

The adverse events were summarized by study treatment, system organ class and preferred term.

Further tables summarized the adverse events by severity, (mild, moderate or severe) and by relationship to study treatment. If a subject experiences the same event more than once, the maximum severity and strongest relationship were used for the summary tables. If severity or relationship are missing these were reported as missing. Serious adverse events and adverse events leading to withdrawal from the study were presented separately.

Full details of all adverse events reported were listed, by treatment, using verbatim and preferred

terms and including the time of onset, period of event, severity, relationship to treatment and outcome. This listing followed the ICH Guidelines (E3) for Clinical Study Reports.

A table or listing was made for those subjects with serious adverse events or events which led to withdrawal from the study.

Vital signs

Vital signs have been summarised as central tendency and dispersion by treatment. Unless data distribution suggests otherwise, data were analysed for possible changes over time by means of Repeated Measure ANOVA, using gender, age and sequence as adjusting factors (Table 3.13 – table 3.15).

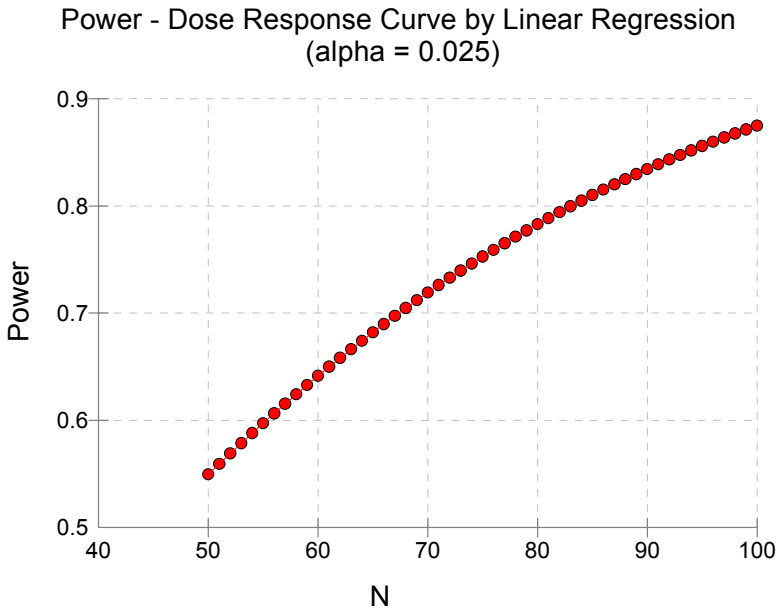
9.7.2. Determination of Sample Size

Primary Objective: Demonstrate a dose-response relationship of OB - 20 mg, 40 mg, 80 mg and placebo administered t.i.d. for 4 weeks - in treatment-sensitive functional variables recorded during the manometric investigations.

The sample size calculation for the study was based on the expected proof of a significant slope of the dose-response curve by linear regression.

The calculations have been performed covering the doses 0 mg (placebo) t.i.d., 20 mg OB t.i.d., 40 mg OB t.i.d. and 80 mg OB t.i.d. Expecting the relation between the standard deviation of the responses and the slope of the dose-response curve not to exceed the value of 250, a sample size of 21 evaluable patients per treatment group (84 evaluable patients) is sufficient to prove significance of the slope at a (one-sided) alpha = 0.025 with the power = 81 %, with 10% drop-out estimated, N = 96.

Figure 1. Sample size.



9.8. CHANGES IN CONDUCT OF THE STUDY OR PLANNED ANALYSES

No protocol amendment was issued during the study and no changes to the planned analyses were made.

10. STUDY SUBJECTS

10.1. DISPOSITION OF SUBJECTS

Patient disposition and patients withdrawing prematurely during the study are shown in Section 14.1 and Appendix 16.2.1. The number of patients before randomisation and during the study and those who discontinued prematurely are displayed, separately for each treatment group, in Tables 4-6.

Table 4. Patient Disposition (All Randomised Patients, N = 93) by treatment.

	Treatment				**Total** (N = 93)
	Placebo (N = 23)	**Otilonium 20 mg** (N = 24)	**Otilonium 40 mg** (N = 23)	**Otilonium 80 mg** (N = 23)	
Total number of patients screened	-	-	-	-	96
Screening failures	-	-	-	-	3 (3.1)
Randomised subjects	23	24	23	23	93
Completed the study [1]	21 (91.3)	24 (100.0)	23 (100.0)	22 (95.7)	90 (96.8)
Discontinued the study [1]	2 (8.7)	-	-	1 (4.3)	3 (3.2)
Withdrawal of consent [2]	2 (100.0)	-	-	-	2 (66.7)
Laboratory abnormalities	-	-	-	1 (100.0)	1 (33.3)

[1] Percentage are based on the number of patients randomised
[2] Percentage are based on the number of patients that discontinued the study

Table 5 Patient Disposition by Study Centre (All Randomised Patients, N = 93). – Continuing

	Treatment				**Total**
	Placebo (N = 23)	**Otilonium 20 mg** (N = 24)	**Otilonium 40 mg** (N = 23)	**Otilonium 80 mg** (N = 23)	**N = 93**
Centre 1					
Randomised subjects	5	6	5	5	21
Completed the study [1]	5 (100.0)	6 (100.0)	5 (100.0)	5 (100.0)	21 (100.0)
Centre 2					
Randomised subjects	6	6	6	6	24
Completed the study [1]	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	24 (100.0)
Centre 3					
Randomised subjects	3	3	3	3	12
Completed the study [1]	2 (66.7)	3 (100.0)	3 (100.0)	2 (66.7)	10 (83.3)
Discontinued the study [1]	1 (33.3)	-	-	1 (33.3)	2 (16.7)
Withdrawal of consent [2]	1 (100.0)	-	-	-	1 (50.0)
Laboratory abnormalities	-	-	-	1 (100.0)	1 (50.0

[1] Percentage are based on the number of patients randomised for any centre
[2] Percentage are based on the number of patients that discontinued the study

Table 6. Patient Disposition by Study Centre (All Randomised Patients, N = 93).

	Treatment				**Total**
	Placebo (N = 23)	**Otilonium 20 mg** (N = 24)	**Otilonium 40 mg** (N = 23)	**Otilonium 80 mg** (N = 23)	**N = 93**
Centre 4					
Randomised subjects	9	9	9	9	36
Completed the study [1]	8 (88.9)	9 (100.0)	9 (100.0)	9 (100.0)	35 (97.2)
Discontinued the study [1]	1 (11.1)	-	-	-	1 (2.8)
Withdrawal of consent [2]	1 (100.0)	-	-	-	1 (100.0)

[1] Percentage are based on the number of patients randomised for any centre
[2] Percentage are based on the number of patients that discontinued the study

A total 96 patients entered the screening phase of the study and 93 (96.8%) were randomised to treatment. The number of patients completing the study was 90, corresponding to 96.8% of the randomised patients. The percentage of patients completing the study for each treatment group was 91.3%, 100%, 100% and 95.7% for placebo, OB 20 mg, OB 40 mg and OB 80 mg respectively. The reasons for dropping-out from the study were laboratory abnormality (n=1) and withdrawal of consent (n=2).

Number of patients performing ano-rectal investigation at baseline (visit 0) and end of 4 weeks treatment period (visit 4) were in total n° 86 (92.5%), of which n° 20 (21.5%), n° 23 (24,7%), n° 23 (24,7%) and n° 20 (21.5%) for placebo, OB 20 mg, OB 40 mg and OB 80 mg respectively.

10.2. PROTOCOL DEVIATIONS

No protocol violations occurred during study realisation.

11. EFFICACY EVALUATION

11.1. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

11.1.1. Demographic Characteristics

Demographic data, including concomitant diseases and treatments, for the ITT are summarised in Section 14.1 and are fully detailed in Appendix 16.2.4. As shown in Text Table 7, no overt differences were observed for baseline demographic data among treatment groups.

Table 7. Demographic and clinic characteristic at screening visit (ITT population).

	Treatment				**Total**
	Placebo (N = 23)	**Otilonium 20 mg** (N = 24)	**Otilonium 40 mg** (N = 23	**Otilonium 80 mg** (N = 23)	**N = 93**
Age (Years)					
Mean (SD)	47.8 (13.11)	44.0 (12.71)	44.6 (13.66)	42.9 (11.12)	44.8 (12.60)
Median	50.0	41.6	46.6	41.9	45.6
Min - max	22.9 – 65.3	22.4 – 64.4	24.3 – 65.8	19.3 – 58.1	19.3 – 65.8
Height (cm)					
Mean (SD)	166.4 (7.90)	170.4 (9.65)	166.1 (7.75)	170.2 (10.92)	168.0 (9.24)
Median	165.0	172.0	165.0	167.0	167.0
Min - max	155.0 – 185.0	152.0 – 194.0	151.0 – 181.0	156.0 – 192.0	151.0 – 194.0
Weight (Kg)					
Mean (SD)	69.8 (15.90)	72.9 (16.63)	68.9 (12.85)	74.4 (16.89)	71.5 (15.56)
Median	69.8	72.0	70.0	69.6	71.0
Min - max	46.6 – 98.0	45.4 – 112.0	51.0 – 90.0	51.9 – 110.0	45.4 – 112.0
Gender					
Male	7 (30.4)	8 (33.3)	7 (30.4)	7 (30.4)	29 (31.2)
Female	16 (69.6)	16 (66.7)	16 (69.6)	16 (69.6)	64 (68.8)
ND	-	-	-	-	-

11.2. MEASUREMENTS OF TREATMENT COMPLIANCE

All the patients were compliant to treatment. Compliance to study treatment resulted in the range ≥80% or ≤120%.Detail can be found in Appendix 16.2.5 and is summarised Section 14.2.

11.3. EFFICACY RESULTS AND TABULATIONS OF INDIVIDUAL PATIENT DATA

11.3.1. Analysis of Efficacy

The primary and secondary efficacy variables were analysed on the ITT and PP population. In the following paragraphs, results of the ITT population are reported, and differences from those of the PP population are discussed when present. Summary tables are detailed in Section 14.2, while individual efficacy results are provided in Appendix 16.2.6.

11.3.1.1. Primary efficacy

Regarding the ITT population, the full dose of the test drug was taken at the end of the four weeks study period by 96.8% of patients.

Text Table 8a and 8b, summarised the efficacy result obtained measuring the induced anatomo-physiologic response of the main bowel parameters measured ano-rectal manometry investigation before and after Otilonium Bromide administration: Pressure of anal sphincter, Eliciting Volume, Volume for first sensation, Volume for desire to defecate, volume for urgency and Volume for discomfort.

Table 8a. Mean values (± SD) of the ano rectal parameters (ITT Population, N = 93).

Parameter	Placebo		OB 20 mg		OB 40 mg		OB 80 mg		Total mean	
	V 0	V 4	V 0	V 4	V 0	V 4	V 0	V 4	V 0	V 4
Length of anal canal	33.7 ± 1.6	32.1 ± 11.7	34.6 ± 8.9	34.6 ± 8.4	33.4 ± 9.8	33.5 ± 11.0	33.6 ± 8.1	33.4 ± 9.0	33.8 ± 9.4	33.4 ± 9.9
Depth of insertion probe	24.8 ± 18.8	27.4 ± 16.9	24.9 ± 14.3	24.8 ± 13.5	24.2 ± 16.7	22.4 ± 16.6	28.8 ± 18.1	24.7 ± 18.3	25.6 ± 16.7	24.7 ± 16.2
Baseline pres. anal sphincter	51.9 ± 19.2	56.8 ± 18.7	50.7 ± 16.4	48.5 ± 17.9	54.4 ± 21.2	49.6 ± 18.2	49.4 ± 20.2	52.9 ± 18.8	51.6 ± 19.0	51.8 ± 18.3

Table 8b Mean values (± SD) of the functional ano rectal parameters (ITT Population, N = 93).

Parameter	Placebo		OB 20 mg		OB 40 mg		OB 80 mg		Total mean	
	V 0	V 4	V 0	V 4	V 0	V 4	V 0	V 4	V 0	V 4
Volume eliciting RAIR	28.2 ± 12.9	31.2 ± 15.4	32.1 ± 15.8	28.2 ± 13.0	23.0 ± 7.3	27.1 ± 14.0	30.5 ± 10.3	24.7 ± 14.7	28.4 ± 12.2	27.7 ± 14.1
Volume for first sensation	32.5 ± 16.5	30.5 ± 16.1	31.5 ± 15.5	30.0 ± 17.6	25.4 ± 11.0	27.9 ± 14.4	35.0 ± 17.6	32.5 ± 14.5	30.9 ± 15.3	30.1 ± 15.3
Volume for desire to defecate	64.5 ± 19.6	56.5 ± 18.7	64.8 ± 20.2	60.0 ± 20.0	57.5 ± 19.2	60.0 ± 16.7	66.0 ± 18.5	63.0 ± 16.3	63.0 ± 19.4	59.9 ± 17.8
Volume for urgency	103.0 ± 35.7	105.0 ± 41.0	108.2 ± 40.8	93.0 ± 33.2	96.3 ± 37.9	102.9 ± 37.5	111.0 ± 32.8	95.0 ± 26.7	104.3 ± 36.8	98.9 ± 34.8
Volume for discomfort	140.0 ± 37.1	140.0 ± 38.9	152.2 ± 40.3	153.0 ± 33.9	153.3 ± 45.2	149.2 ± 35.9	144.4 ± 36.7	151.0 ± 37.0	148.1 ± 40.0	148.6 ± 36.0
Mean squeeze pressure	109.9 ± 35.0	112.5 ± 46.3	126.9 ± 38.2	117.0 ± 40.8	120.1 ± 27.0	117.9 ± 39.3	100.9 ± 34.7	116.1 ± 36.1	114.7 ± 35.4	115.1 ± 39.8
Squeeze time	19.5 ± 1.9	20.0 ± 0.0	19.9 ± 0.2	20.0 ± 0.0	20.0 ± 0.0	20.0 ± 0.0	19.8 ± 0.8	20.0 ± 0.0	19.8 ± 1.0	20.0 ± 0.0

Table 9. Rectum compliance after air inflation. Mean (± SD) (ITT Population, N = 93).

Rectum compliance	Placebo		OB 20 mg		OB 40 mg		OB 80 mg		Total mean	
	V 0	V 4	V 0	V 4	V 0	V 4	V 0	V 4	V 0	V 4
20 ml inflation	50.5 ± 16.9	42.5 ± 11.1	48.1 ± 21.7	44.6 ± 13.9	46.8 ± 17.9	41.1 ± 9.6	49.1 ± 16.2	46.2 ± 12.2	48.5 ± 18.2	43.5 ± 11.7
40 ml inflation	51.3 ± 41.9	44.6 ± 13.9	49.3 ± 43.6	42.7 ± 8.6	46.0 ± 18.1	38.7 ± 7.2	49.2 ± 17.4	42.4 ± 9.9	48.7 ± 31.8	41.9 ± 10.0
60 ml inflation	49.4 ± 35.8	43.7 ± 14.4	43.5 ± 28.6	40.6 ± 6.9	47.2 ± 16.3	40.3 ± 7.6	43.8 ± 15.0	40.3 ± 9.0	45.9 ± 24.6	41.2 ± 9.6
80 ml inflation	41.0 ± 12.4	44.5 ± 15.7	40.5 ± 24.1	37.0 ± 6.3	40.7 ± 17.6	37.1 ± 9.3	43.4 ± 12.5	39.0 ± 9.1	41.3 ± 17.5	30.1 ± 10.5
100 ml inflation	41.4 ± 10.2	42.2 ± 15.2	43.9 ± 25.3	38.0 ± 7.7	40.6 ± 13.4	37.4 ± 7.8	42.8 ± 16.4	40.0 ± 9.9	42.2 ± 17.1	39.2 ± 10.2
140 ml inflation	51.4 ± 23.0	45.2 ± 14.9	43.9 ± 15.7	42.3 ± 7.7	42.5 ± 12.4	41.1 ± 11.1	41.8 ± 11.0	41.0 ± 8.6	44.5 ± 15.6	42.2 ± 10.5
180 ml inflation	48.2 ± 16.6	47.3 ± 16.8	42.4 ± 19.1	41.0 ± 12.7	39.8 ± 11.4	39.5 ± 10.8	42.6 ± 9.1	48.9 ± 17.6	42.6 ± 13.8	43.6 ± 14.2
200 ml inflation	42.0 ± 1.7	35.0 ± 4.2	29.0 ± 25.5	44.0 -	40.0 ± 15.5	36.3 ± 03.8	39.3 ± 15.3	48.0 -	38.7 ± 14.0	38.7 ± 5.9

As shown in table 8a, 8b and 9, for all the evaluated parameters a large experimental variability was observed. This did not allow the applicability of inferential test appropriate to the evaluation of collected ano-rectal manometry data.

11.3.1.2. Secondary Efficacy

In consequence of the great variability observed in the distribution of the Functional target variable of efficacy the analysis of the Clinical Target Variables of Efficacy was considered as primary objective. The analysis of the Functional Target Variables of Efficacy were completed before the blind opening phase according to the ICH E9, Note for guidance on statistical principles for clinical trail, EMEA CPMP/ICH/363/96.

Table 10. Intensity of abdominal discomfort, bloating or pain. Mean and Δ% values (ITT Population, N = 93).

Study Period			**Placebo** (n=23)	**OB 20 mg** (n=24)	**OB 40 mg** (n=23)	**OB 80 mg** (n=23)
Wash-out	Day -7 / -1 (Baseline)	Mean	2.37	2.04	2.19	2.19
Treatment	Day 1 - 7	Mean - difference from baseline (%)	2.29 -3.4	1.80 -11.8	1.88 -14.2	1.79 -18.3
	Day 8 - 14	Mean - difference from baseline (%) - difference from week 1-7 (%)	2.02 -14.8 -2.0	1.63 -20.1 -9.4	1.91 -12.8 +1.6	1.77 -19.2 -1.1
	Day 15 - 21	Mean - difference from baseline (%) - difference from week 8-14 (%)	1.86 -21.5 -7.9	1.50 -26.5 -8.0	1.67 -23.7 -12.6	1.59 -27.4 -10.2
	Day 22 - 28	Mean - difference from baseline (%) - difference from week 15-21 (%)	1.80 -24.1 -3.2	1.45 -28.9 -3.3	1.65 -24.7 -1.2	1.49 -32.0 -6.3

Table 11. Daily frequency of abdominal discomfort, bloating or pain. Mean and Δ% values (ITT Population, N = 93).

Study Period			**Placebo** (n=23)	**OB 20 mg** (n=24)	**OB 40 mg** (n=23)	**OB 80 mg** (n=23)
Wash-out	Day -7 / -1 (Baseline)	Mean	3.09	3.55	2.79	3.35
Treatment	Day 1 - 7	Mean - difference from baseline (%)	2.84 -8.1	3.43 -3.4	2.43 -12.9	2.77 -14.8
	Day 8 - 14	Mean - difference from baseline (%) - difference from week 1-7 (%)	2.19 -29.1 -22.9	2.66 -25.1 -22.5	2.33 -16.5 -4.1	2.29 -29.5 -17.3
	Day 15 - 21	Mean - difference from baseline (%) - difference from week 8-14 (%)	1.99 -36.6 -9.1	2.69 -24.2 +1.1	2.13 -23.7 -8.6	2.72 -18.8 +18.8
	Day 22 - 28	Mean - difference from baseline (%) - difference from week 15-21 (%)	2.18 -29.5 +9.6	2.72 -23.4 +1.1	1.94 -30.5 -8.9	2.16 -35.5 -20.6

Table 12. Number of evacuations. Mean and Δ% values (ITT Population, N = 93).

Study Period			**Placebo** (n=23)	**OB 20 mg** (n=24)	**OB 40 mg** (n=23)	**OB 80 mg** (n=23)
Wash-out	Day -7 / -1 (Baseline)	Mean	1.86	1.54	2.00	1.77
Treatment	Day 1 - 7	Mean - difference from baseline (%)	1.90 +2.2	1.39 -9.7	1.72 -14.0	1.59 -10.2
	Day 8 - 14	Mean - difference from baseline (%) - difference from week 1-7 (%)	1.77 -4.8 -6.8	1.44 -6.5 +3.6	1.61 -19.5 -6.4	1.62 -8.5 +1.9
	Day 15 - 21	Mean - difference from baseline (%) - difference from week 8-14 (%)	1.64 -11.8 -7.3	1.29 -16.2 -10.4	1.93 -3.5 +19.9	1.39 -21.5 -14.2
	Day 22 - 28	Mean - difference from baseline (%) - difference from week 15-21 (%)	1.70 -8.6 +3.7	1.36 -11.7 +5.4	1.63 -18.5 -15.5	1.12 -36.7 -19.4

Table 13. Mucus, sensation of incomplete evacuation and difficulty of evacuation. Mean and Δ% values (ITT Population, N = 93).

Study Period			**Placebo** (n=23)	**OB 20 mg** (n=24)	**OB 40 mg** (n=23)	**OB 80 mg** (n=23)
Wash-out	Day -7 / -1 (Baseline)	Mean	2.56	2.22	2.45	2.33
Treatment	Day 1 - 7	Mean - difference from baseline (%)	2.49 -2.7	2.31 +4.1	2.32 -5.3	2.47 +6.0
	Day 8 - 14	Mean - difference from baseline (%) - difference from week 1-7 (%)	2.52 -1.6 +1.2	2.53 +14.0 +9.5	2.41 -1.6 +3.9	2.45 +5.2 -0.8
	Day 15 - 21	Mean - difference from baseline (%) - difference from week 8-14 (%)	2.55 -0.4 +1.2	2.27 +2.3 -10.3	2.93 +19.6 +21.6	2.40 +3.0 -2.0
	Day 22 - 28	Mean - difference from baseline (%) - difference from week 15-21 (%)	2.43 -5.1 -4.7	2.47 +11.3 +8.8	2.70 +10.2 -7.9	2.43 +4.3 +1.3

Text Tables from 10 to 13, summarise the efficacy result obtained measuring the clinical variables of efficacy reordered by patient through in the weekly diary. As described in section 9.6 the “intensity and frequency of abdominal discomfort or pain”, the “intestinal habits”; the “number of evacuations and the days without evacuation” and “Mucus in stool incomplete or difficult in evacuation were scored using appropriate scales.

Regular Stool Index

Table 14. Consistency and shape of the stool. Mean and Δ values (ITT Population, N = 93).

Study Period			**Placebo** (n=23)	**OB 20 mg** (n=24)	**OB 40 mg** (n=23)	**OB 80 mg** (n=23)
Wash-out	Day -7 / -1 (Baseline)	RSR	32.2	25.8	35.8	30.0
Treatment	Day 1 - 7	RSR - difference from baseline	35.4 + 3.2	33.3 + 7.5	44.1 + 8.3	41.6 + 11.6
	Day 8 - 14	RSR - difference from baseline - difference from week 1-7	42.2 + 10.0 + 6.8	37.5 + 11.7 + 4.2	41.7 + 5.9 - 2.4	39.6 + 9.6 - 2.0
	Day 15 - 21	RSR - difference from baseline - difference from week 8-14	47.2 + 15.0 + 5.0	38.7 + 12.9 + 1.2	51.8 + 16.0 + 10.1	41.0 + 11.0 + 1.4
	Day 22 - 28	RSR - difference from baseline - difference from week 15-21	47.8 + 15.6 + 0.6	41.1 + 15.3 + 2.4	45.2 + 9.4 - 6.6	46.7 + 16.7 + 5.7

Differently (see table14) in order to evaluate the "consistency and the shape of the stool", the Bristol classification was used and the quantitative index named RSR (Regular Stool Rate) was create. RSR was calculating according the formula:

RSR = Number of day with Regular Stool／Total number of day ·● 100

Global Discomfort Index

In order to evaluate the clinical efficacy data as a whole, a general index called: "Global Index discomfort (GDI)" was created. The GDI index is calculated according the following formula:

$$GDI = \frac{\text{(Daily frequency of the abdominal discomfort, bloating or pain) * (Number of evacuations)}}{\text{GDI Mean (Screening Period)}} * 100$$

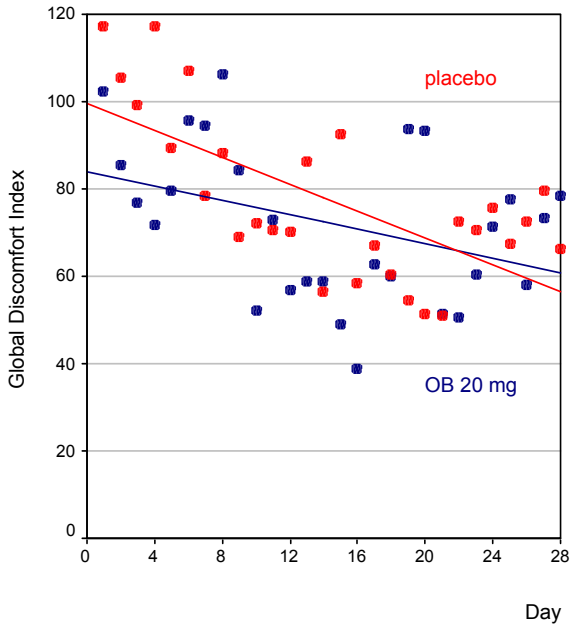
Where:
1 GDI Mean = Mean of the Efficacy Index in the 14 days before randomization (Screening Period).

In the following figure 2 (a-d) is graphically depicted the comparisons among GDI index computed for Placebo treatment group with groups treated with OB 20, 40 and 80 mg respectively (Fig. 2a - fig 2c). Fig 2d shows the direct comparison between OB 40mg and OB80 mg groups.

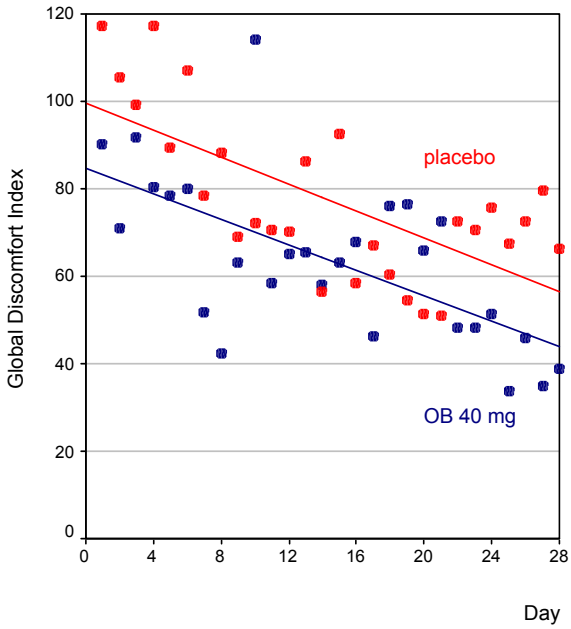
Statistical result of these comparisons are described in table N° 15 Man-Whitney and Wilcoxon non parametric tests were applied. In comparisons to the Placebo group, results shown a significant reduction of the GD Index values for group treated with OB 40 mg ($p<0.01$) and for group treated with OB 80 mg ($p< 0.001$). No significant difference was observed for Placebo vs. 20 mg OB group and 40 mg OB vs. OB 80 mg groups.

Figure 2. Global discomfort index (from day 1 to day 28).

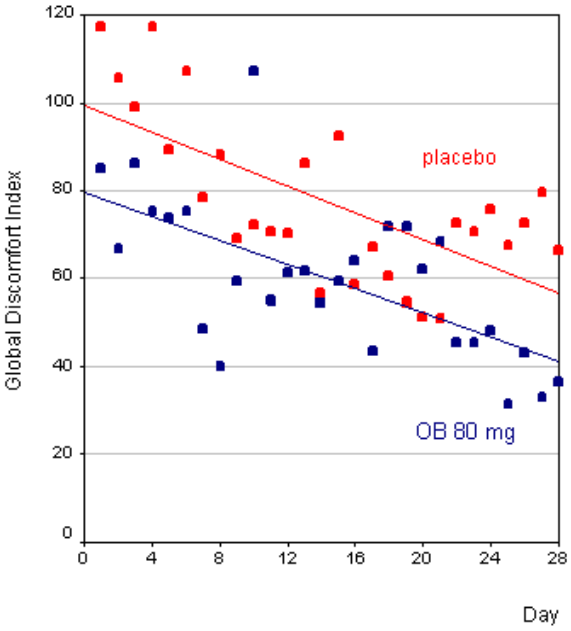
Placebo vs. OB 20 mg (2a)



Placebo vs. OB 40 mg (2b)



Placebo vs. OB 80 mg (2c)



OB 40 mg vs. OB 80 mg (2d)

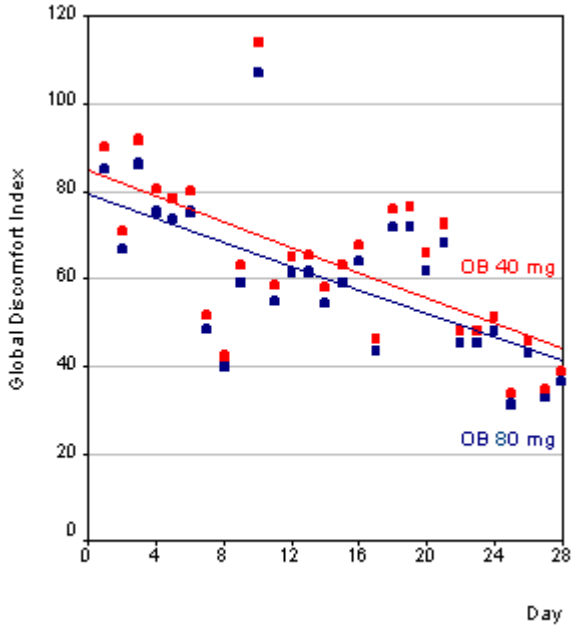


Table 15. Global discomfort index (from day 1 to day 28). Mann-Whitney and Wilcoxon test.

Treatment	Ranks		Non Parametric Tests			
	Mean Rank	Sum of Ranks	Mann-Whitney U	Wilcoxon	Z	P
placebo vs. OB 20 mg	30.2	845.0	345.0	751.0	-0.77	0.441
	26.8	751.0				
placebo vs. OB 40 mg	33.89	949.00	241.0	647.0	-2.47	**0.013**
	23.11	647.00				
placebo vs. OB 80 mg	35.61	997.00	193.0	599.0	-3.26	**0.001**
	21.39	599.00				
OB 40 mg vs. OB 80 mg	30.39	851.00	339.0	745.0	-0.87	0.385
	26.61	745.00				

Intestinal habits

Data relative to "Intestinal habits" were analysed classifying study population (ITT population n° = 93) in three groups. Subject having "regular habits", subject suffering of "Constipation" and subjects suffering of "Diarrhoea".

"Regular "intestinal habits

Results on "Regular" intestinal habits defined as: "All the conditions not present like constipation or diarrhoea or alternating or more then 2 evacuations during the week", are shown in figure n°3 and table 16.
The subjects belonging to treated groups met an improvement and a normalization of their intestinal conditions. The particular an improvement of regular "intestinal habits" was observed for the 41.7% ($p < 0.01$) and 35.0% of the patients treated with 40 mg and 80 OB respectively.

Figure 3. Increase (%) of the subjects with **Regular Intestinal Habits**[1] by Study Visit.

[1] Regular Intestinal Habits: All the conditions not present like constipation or diarrhoea or alternating or more then 2 evacuations during the week.

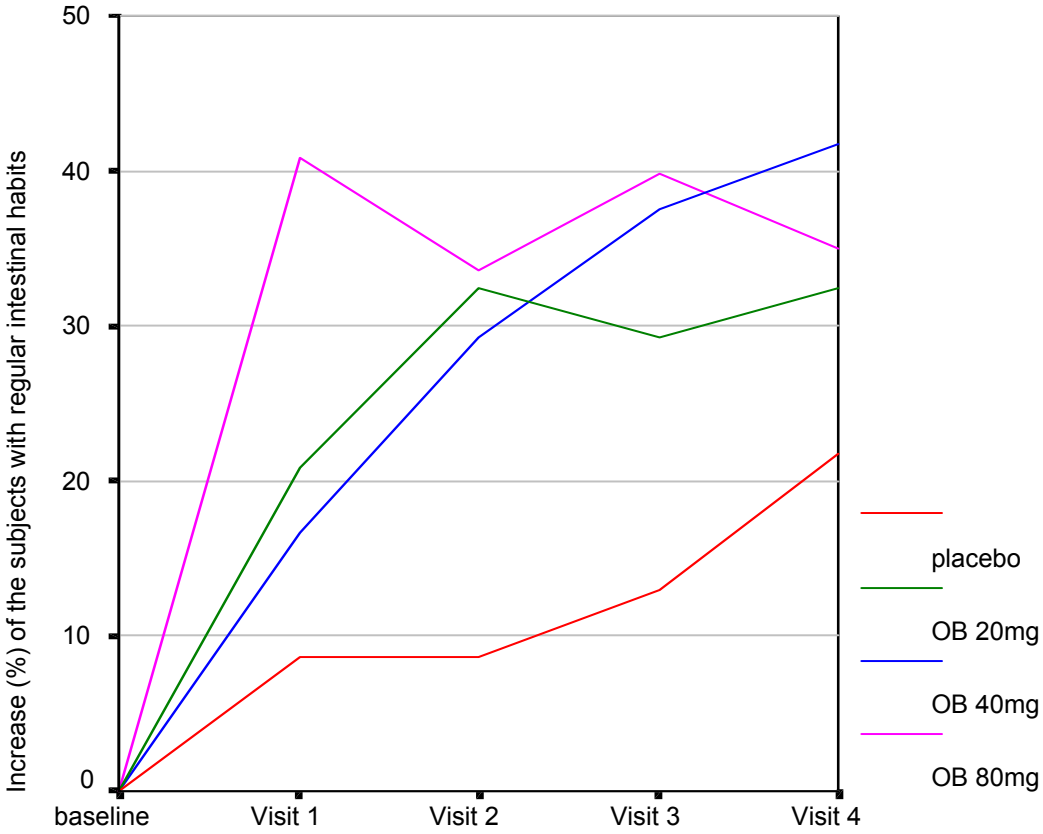


Table 16. Percentage (%) of subjects with "**REGULAR**" Intestinal Habits by Study Visit (ITT Population, N = 93).

Treatment	**Percentage (%) of subjects with "REGULAR" Intestinal Habits**						
	Visit -1 (week -1)	**Visit 0** (week 0)	**Visit 1** (week 1)	**Visit 2** (week 2)	**Visit3** (week 3)	**Visit 4** (week 4)	**Difference V4 - V0**
placebo (N = 23)	52.2	52.2	60.9	60.9	65.2	73.9	+ 21.7
OB 20 mg (N = 24)	30.4	45.8	66.7	78.3	75.0	78.3	+ 32.5
OB 40 mg (N = 23)	45.8	37.5	54.2	66.7	75.0	79.2	+ 41.7
OB 80 mg (N = 23)	27.3	36.4	77.3	70.0	76.2	71.4	+ 35.0

Stool frequency by treatment

Within the parameter "shape and consistency of the stool", classified according to the Bristol Index, the higher frequency of patients with “normal stool consistency” was observed in the group treated with OB 80 mg. In this group was also found the lowest percentage of subjects with constipation (13%) and diarrhoea (30.4%). See figure 4 a-b and table 17.

Figure 4. Stool Frequency[1] by Treatment – (Week 1, Day 1-7), (ITT Population, N = 93).

[1] Stool Frequency Groups definition:

Group **A**: Stool Frequency ≤ 3 / week (Constipation)
Group **B**: Stool Frequency > 3 / week and < 3 / day (Normal)
Group **C**: Stool Frequency ≥ 3 / day (Diarrhoea)

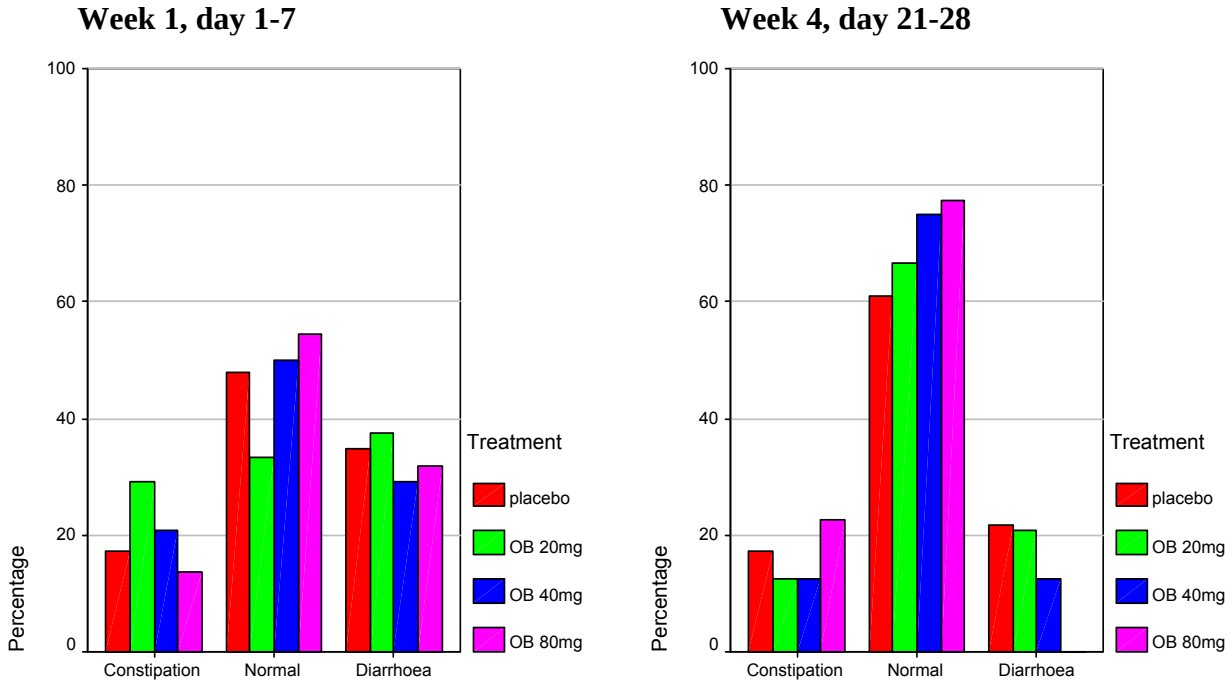


Table 17. Stool Frequency by Treatment – (Week 1, Day 1-7), (ITT Population, N = 93).

	Week 1 % within treatment					Week 4 % within treatment				
	Placebo	**OB 20mg**	**OB 40mg**	**OB 80mg**	**Total**	**Placebo**	**OB 20mg**	**OB 40mg**	**OB 80mg**	**Total**
Constipation	17.4%	29.2%	21.7%	13.0%	20.4%	17.4%	12.5%	13.0%	21.7%	16.1%
Normal	47.8%	33.3%	47.8%	56.5%	46.2%	60.9%	66.7%	73.9%	78.3%	69.9%
Diarrhoea	34.8%	37.5%	30.4%	30.4%	33.3%	21.7%	20.8%	13.0%	0.0%	14.0%

11.3.2. Statistical/Analytical Issues

Full details of the statistical analyses are presented in Appendix 16.1.9. Discussion of some relevant issues related to statistical analysis are briefly summarised below.

11.3.3. Tabulation of Individual Response Data

Appendix 16.2.6 shows the individual patient data.

11.3.4. Drug Dose, Drug Concentration and Relationships to Response

Not applicable.

11.3.5. Drug-Drug and Drug-Disease Interactions

No formal statistical analyses of drug-drug and drug-disease interactions were performed.

11.3.6. By-Patient Displays

Not applicable.

11.3.7. Efficacy Conclusions

Analysis of the primary efficacy variables (ano-rectal manometry investigation before and after Otilonium administration) did not show significantly differences between the treatment groups. This was due to the large experimental variability observed.
In consequence of the great variability observed in the distribution of the Functional target variable of efficacy the analysis of the Clinical Target Variables of Efficacy was considered as primary objective.
This objective was performed measuring the clinical variables of efficacy recorded by patient through in the weekly diary. In particular the “intensity and frequency of abdominal discomfort or pain”, the “intestinal habits”; the “number of evacuations and the days without evacuation” and “Mucus in stool incomplete or difficult in evacuation were scored using value scales.
The analysis of these collected data showed that the groups of patients treated with OB 40 mg and 80 mg were significantly different from patients group treated with Placebo or OB 20 mg.
Otherwise, no significant difference was observed comparing the group treated with OB 80 mg with the group treated with and 40 mg OB.

In conclusion results shown a significant reduction of the GD Index values for group treated with OB 40 mg ($p<0.01$) and for group treated with OB 80 mg ($p< 0.001$). No significant difference was observed for Placebo vs. 20 mg OB group and 40 mg OB vs. OB 80 mg groups. Therefore we can say that, in patients suffering of IBS, the treatment with Otilonium Bromide (OB 40 mg in particular), can lead to an improvement of clinical parameters such as: abdominal discomfort, intestinal habits, number of daily evacuations and stool consistency.

12. SAFETY EVALUATION

12.1. EXTENT OF EXPOSURE

The safety population included all patients who had received at least one dose of active study medication.

No major differences in the mean, or distribution of the duration of exposure to study treatment were observed between treatment groups (Table 18).

12.1.1. Table 18. Extent of Exposure[1] (Safety Population, N = 93).

Extent of Exposure (days)	Treatment				Total N = 93
	Placebo N = 23	**OB 20 mg** N = 24	**OB 40 mg** N = 23	**OB 80 mg** N = 23	
Mean (SD)	29.7 3.07	29.2 2.64	30.0 3.50	28.7 4.69	29.4 3.54
Median	29.0	28.5	29.0	29.0	29.0
Min - Max	26.0 – 39.0	26.0 – 34.0	26.0 – 39.0	11.0 – 37.0	11.0 – 39.0

[1] Difference between the date of first study drug intake and the date of last study drug intake.

Data source: Section 14.3 and Appendix 16.2.7.

12.2. ADVERSE EVENTS (AES)

12.2.1. Display of Adverse Events

Emerged Adverse events (AEs) are presented in Appendix 16.2.7 and summarised in Section 14.3. An overview of AEs reported during the study is listed by patient in Table 19.

Table 19. Number of all AEs information by treatment (Safety population).

Centre	Random	OB Treatment (mg)	Adverse Event	Start data	End data	Intensity	Serious	Outcome	Relation
1	6	20 mg	Pneumonia	01/01/2008	16/01/2008	2	No	Resolved	Not related
1	8	40 mg	Flu fever, (common cold syndrome)	11/12/2007	14/12/2007	1	No	Resolved	Not related
1	9	40 mg	Asthma exacerbation	10/01/2008	10/01/2008	1	No	Resolved	Not related
1	9	40 mg	GI Infection (Diarrhoea, fever)	13/01/2008	18/01/2008	2	No	Resolved	Not related
1	12	80 mg	Cystitis	21/12/2007	08/01/2008	1	No	Resolved	Not related
1	12	80 mg	Migraine Attack - nausea	26/12/2007	26/12/2007	1	No	Resolved	Not related
1	56	Placebo	Flu	19/04/2008	30/04/2008	1	No	Resolved	Not related
1	59	20 mg	Headache	21/05/2008	25/05/2008	1	No	Resolved	Possible related
1	11	80 mg	Nausea	23/05/2008	26/05/2008	1	No	Resolved	Possible related
1	58	20 mg	Xerostomie	09/05/2008	14/05/2008	2	No	Resolved	Unlikely related
2	69	20 mg	High fever (Runny nose, coughing, lacrimation)	04/04/2008	04/04/2008	1	No	Resolved	Not related
2	65	Placebo	Serious headache	27/03/2008	29/03/2008	2	No	Resolved	Not related
3	30	20 mg	Pharyngitis	03/03/2008	09/03/2008	2	No	Resolved	Not related
4	76	Placebo	Headache	21/02/2008	03/03/2008	2	No	Resolved	Probably related

12.2.2. Analysis of Adverse Events

In the following table are summarized the emerged AE.

Table 20. Summary of the emerged Adverse event related to study drug by "treatment and Intensity" (Safety population).

	Adverse Events related to study drug (N°= 4)		
	Mild	Moderate	Severe
Total number of related AEs (N)	2 (50.0)	2 (50.0)	-
Placebo	-	1 (50.0)	-
Otilonium Bromide 20 mg - N[1] (%)	1 (50.0)	1 (50.0)	-
Otilonium Bromide 40 mg - N[1] (%)	-	-	-
Otilonium Bromide 80 mg - N[1] (%)	1 (50.0)	-	-

[1] %: denominator = N by intensity group

Data source: Section 14.3 and Appendix 16.2.7.

12.2.3. Listing of Adverse Events by Patient

All AEs for each patient are shown in Appendix 16.2.7.

12.3. DEATHS, OTHER SERIOUS ADVERSE EVENTS AND OTHER SIGNIFICANT ADVERSE EVENTS

12.3.1.1. Deaths

No deaths occurred.

12.3.1.2. Other Serious Adverse Events

No SAE occurred.

12.4. SAFETY CONCLUSIONS

The number of patients experiencing AEs was low. Most of the AE were of a mild intensity and were not drug related.
In conclusion, tolerability of Otilonium Bromide was good and comparable to that of Placebo group.

13. DISCUSSION AND OVERALL CONCLUSIONS

Although the pathogenesis of IBS is not fully understood, it appears that several factors may be involved. IBS symptoms originate as a response to disruption of GI function secondary due to infection, dietary factors, lifestyle changes or psychological stress, abnormal GI motility, visceral hypersensitivity, autonomic activity. CNS modulation and inflammation have all been implicated as having important roles in the development of IBS.
Epidemiological data indicate that also the psychosocial factors play an important role in the pathophysiology of this disease.
While a part of IBS patients doesn't show any psychopathological signs, a huge number show features of formal psychiatric illness or personality disorders. Moreover many patients have a history of social stress that coexists or exacerbates pre-existing psychological disorders.
It also known that, in patients suffering of IBS, an enhanced perception of visceral events occurs throughout the entire GI tract is present. according with this idea, individuals with IBS are more likely to be aware of intestinal contractions and gas. A weak correlation between pain threshold and the degree of clinical pain experienced has also been reported in IBS patients. In addition, pain is experienced by IBS patients at lower volumes and pressures when a balloon is inflated in their colon. Although there is contradicting evidence regarding somatic sensitivity in IBS, enhanced somatic sensitivity to pain is not seen in IBS patients, and they may even have elevated thresholds to somatic pain.
Many confounding factors may therefore appear during a clinical study on functional disorders such as IBS and complicate the evaluation of experimental findings. To avoid this, on the present study was applied a study design that included for all treatment groups a run-in period of 2 weeks, in order to define baseline IBS values and one experimental group treated with placebo. Moreover we introduced the use of an index, called "GDI" (Global discomfort Index), which allowed us to calculate a value summarizing the data of abdominal discomfort, bloating or pain and N°. of evacuation collected during the study and the run-in period.
After the analysis of study results, the primary efficacy variables (ano-rectal manometry investigation before and after Otilonium administration) did not show any significantly differences between the treatment groups. This was due to the large experimental variability observed.
In consequence of the great variability observed in the distribution of the functional target variables of efficacy, the analysis of the Clinical Target Variables of Efficacy was considered as primary objective.
This objective was performed measuring the clinical variables of efficacy recorded by patient through in the weekly diary. In particular the "intensity and frequency of abdominal discomfort or pain", the "intestinal habits"; the "number of evacuations and the days without evacuation" and "Mucus in stool incomplete or difficult in evacuation were scored using value scales.
The analysis of these data showed that the groups of patients treated with OB 40 mg and 80 mg were significantly different from patients group treated with Placebo or OB 20 mg.
Otherwise, no significant difference was observed comparing the group treated with OB 80 mg with the group treated with and 40 mg OB.
The treatment with OB in the range from 20 to 80 mg TID was well tolerated. No SAE occurred during the study period. A significant reduction of the GD Index values was observed in the group treated with OB 40 mg ($p<0.01$) and with OB 80 mg ($p< 0.001$). No significant difference was observed for Placebo vs. 20 mg OB group and for 40 mg OB vs. OB 80 mg groups.
In conclusion we can say that, in patients suffering of bowel irritable syndrome, the treatment with Otilonium Bromide and OB 40 mg in particular, can lead to an improvement of clinical parameters such as: abdominal discomfort, intestinal habits, number of daily evacuations and stool consistency.

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15. APPENDICES

The following appendices are included:

16.1 Study Information

16.1.1 Protocol And Protocol Amendments

16.1.2 Sample Case Report Form (unique pages only)

16.1.3 List Of Independent Ethics Committees Or Institutional Review Boards - Representative Written Information For Patient And Sample Consent Forms

16.1.4 List Of Investigators And Other Important Participants

16.1.5 Signatures Of Principal Or Co-ordinating Investigator(s) Or Sponsor's Responsible Medical Officer

16.1.6 Listing Of Patients Receiving Test Drug(s)/Investigational Product(s) From Specific Batches Where More Than One Batch Was Used

16.1.7 Randomisation Scheme And Codes

16.1.8 Audit Certificates

16.1.9 Documentation Of Statistical Methods

16.1.10 Documentation Of Inter-Laboratory Standardisation Methods And Quality Assurance Procedures

16.1.11 Publications Based On The Study

16.1.12 Important Publications Referenced In The Report

16.2 Patient Data Listings

16.2.1 Discontinued Patients

16.2.2 Protocol Deviations

16.2.3 Patients Excluded From The Efficacy Analysis

16.2.4 Demographic Data

16.2.5 Compliance And/Or Drug Concentration Data

16.2.6 Individual Efficacy Response Data

16.2.7 Adverse Event Listings

16.2.8 Listing Of Individual Laboratory Measurements By Patient

16.2.9 Vital signs

16.3 Case Report Forms

16.3.1 Case Report Forms For Deaths, Other Serious Adverse Events And Withdrawals For Adverse Events

16.3.2 Other Case Report Forms Submitted